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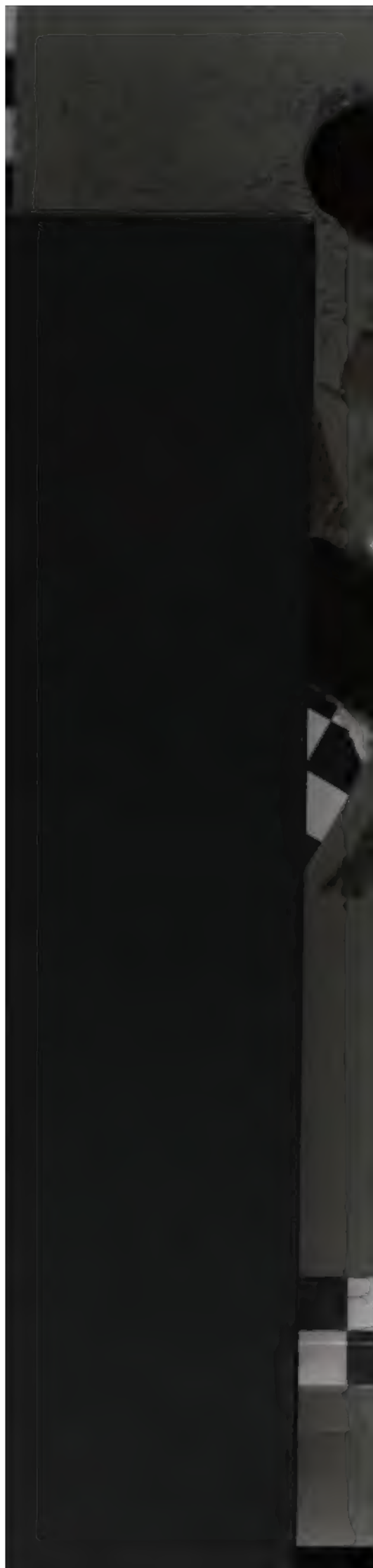
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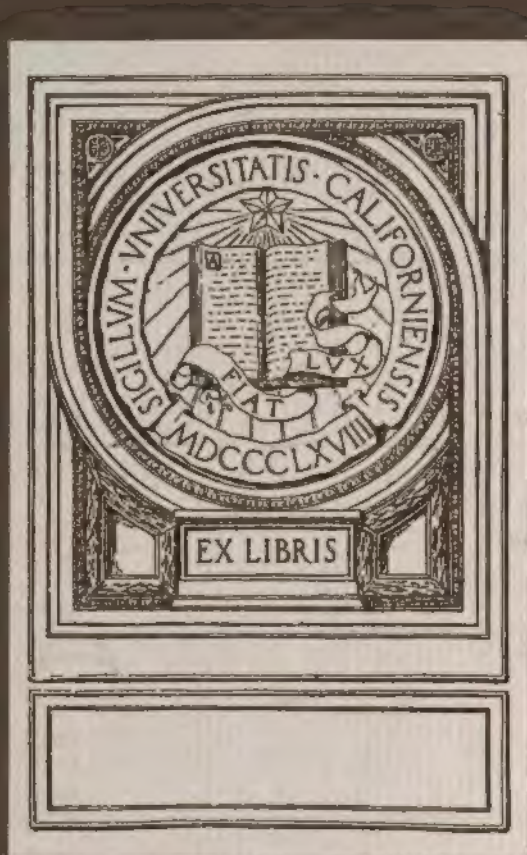
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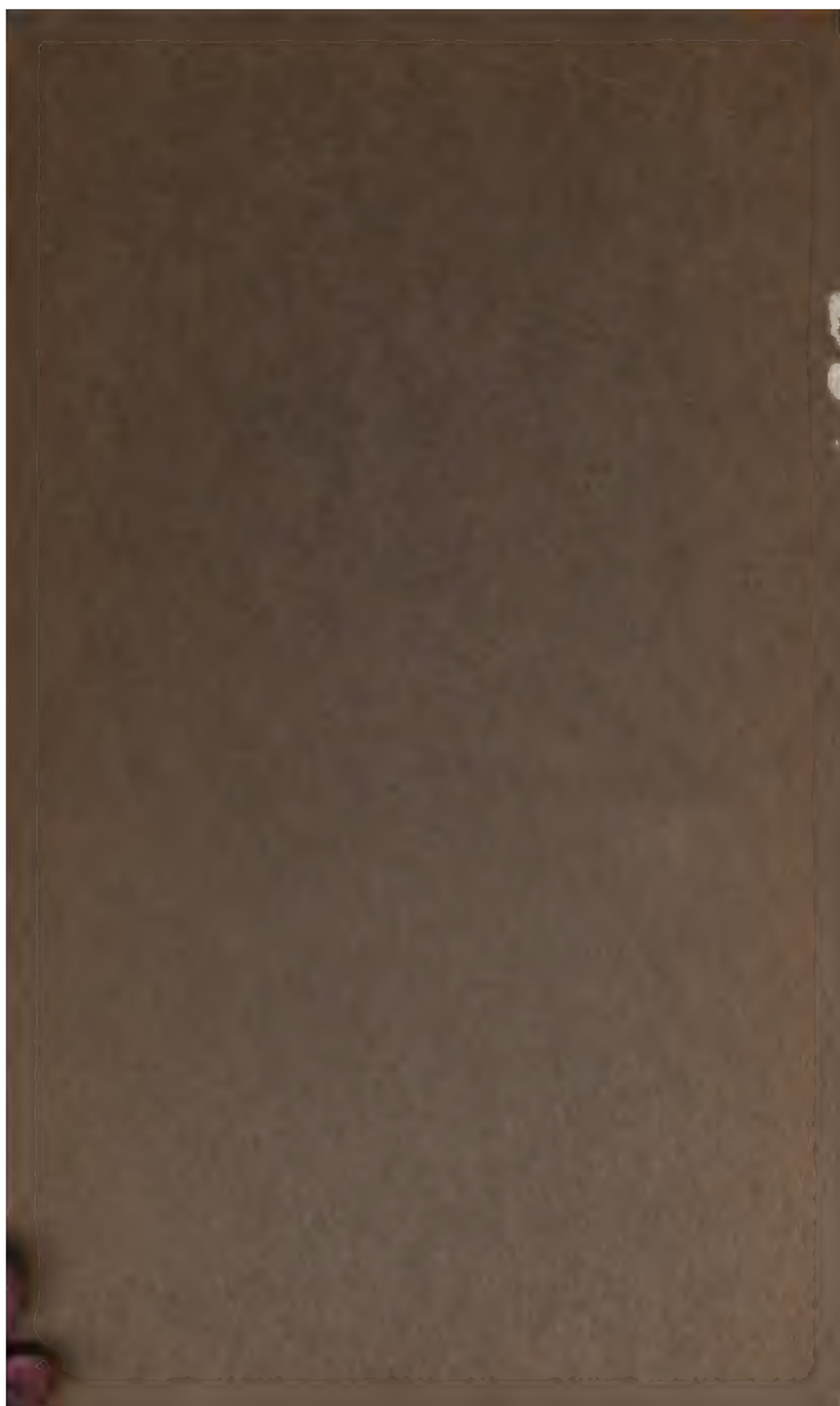
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THE

AMERICAN NATURALIST

UNIV. OF
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A MONTHLY JOURNAL DEVOTED TO THE
NATURAL SCIENCES IN THEIR
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REPRINT

FROM

VOL. XXXVI, No. 426. JUNE, 1902

BOSTON

GINN & COMPANY

The Athenæum Press

1902

to the
American

THE AMERICAN NATURALIST

VOL. XXXVI.

June, 1902.

No. 426.

THE LIFE HISTORY OF *ULULA HYALINA* LATREILLE.¹

J. F. McCLENDON.

WHILE collecting insects in Galveston, Texas, during June, 1900, I found specimens of *Ulula hyalina* Latreille, and at the suggestion of Dr. Wheeler kept them alive for the purpose of obtaining their eggs and following their larval and pupal development. The insects would fly against the sides of the large glass jar in which they were kept, and finally killed themselves; but two females deposited their eggs before they died. After many of the eggs and young larvæ were destroyed by various accidents, I finally succeeded in raising two larvæ. One of these I preserved when full-grown, the other after it had pupated. Later I obtained several imagoes from different localities and one full-grown larva from Austin, Texas. After reading the few notes that have been published on the life history of this interesting insect, I concluded that a more thorough treatment of the subject would not be out of place.

The first notes on the life history of the Ascalaphidæ, the family to which our insect belongs, were published in 1826

¹ *Contributions from the Zoölogical Laboratory of the University of Texas*, No. 27.

by Guilding, who is perhaps best known to zoölogists as the discoverer of *Peripatus*. These notes were on the life history of *Ulula macleayanus* Guilding,¹ which is synonymous with *Ulula hyalina*, or a variety of it, occurring in the island of St. Vincent, West Indies, where Guilding obtained his specimens.

His description is meager and, to some extent, erroneous; but the arrangement of the repagula has not been observed subsequently to my knowledge. Hagen published a paper² in 1873,

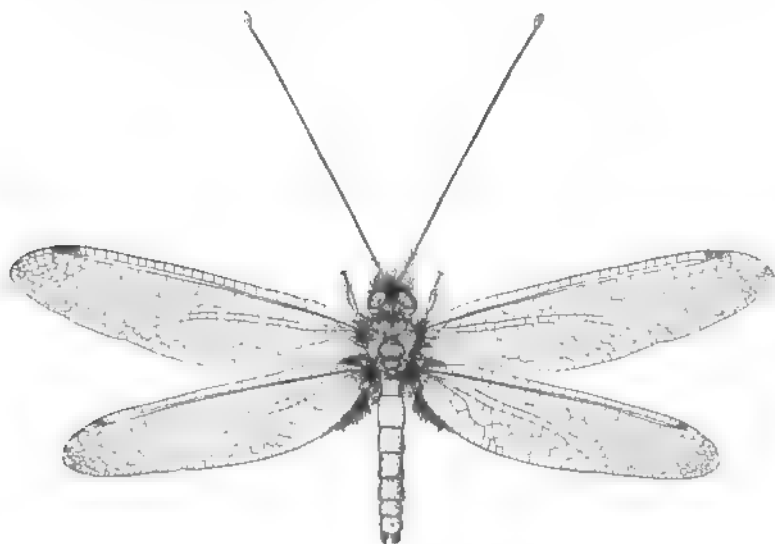


FIG. 1. — *Ulula hyalina* Latr. Male.

which contained short descriptions of sixteen species of Ascalaphidæ. He repeated Guilding's descriptions and also described the larva of *Ulula senex* Burm., ignoring the fact that it was synonymous with *Ulula hyalina* and, consequently, also with *U. macleayanus*. Westwood wrote a review in 1888 of the previous papers on the life history of the Ascalaphidæ³ and added a short life history of a Ceylonese species, perhaps *Ascalaphus insimulans* Walk., accompanied by figures, but

¹ The Genus *Ascalaphus*, *Transactions Linnæan Society*, vol. xv, p. 509.

² Die Larven von *Ascalaphus*, *Stettiner entomologische Zeitung*, Jahrg. xxxiv (1873), p. 33.

³ Notes on the Life History of Various Species of the Neuropterous Genus *Ascalaphus*, *Transactions Entomological Society*, London, 1888, pp. 1-12, Pls. I, II.

failed, as did Guilding and Hagen, to work out the mouth parts thoroughly. A good description of the ant-lion's mouth parts, which are very similar to those of the ascalaphid larva, may be found in a paper by Redtenbacher¹ as early as 1884. Hagen and Westwood do not appear to have been familiar with this paper.

Ulula hyalina is distributed over the southern half of the United States, Mexico, and the West Indies, but is comparatively rare in many of the places where it is found. The insect (Fig. 1) when at rest remains motionless on some small branch or stalk, head down, with wings and antennæ closely applied to the branch, and abdomen erected and often bent so as to resemble a short brown twig or dried branch. On being approached, the insect moves to the opposite side of the branch, and, on being further disturbed, flies to another branch and alights with head up, then quickly turns and assumes its characteristic attitude. I found specimens on stalks of green sedge near the beach at Galveston, Texas. The insect contrasted strongly with the green stalk, but there were, near the tops of the stalks, brown seeds which resembled the insect and made it hard to find.

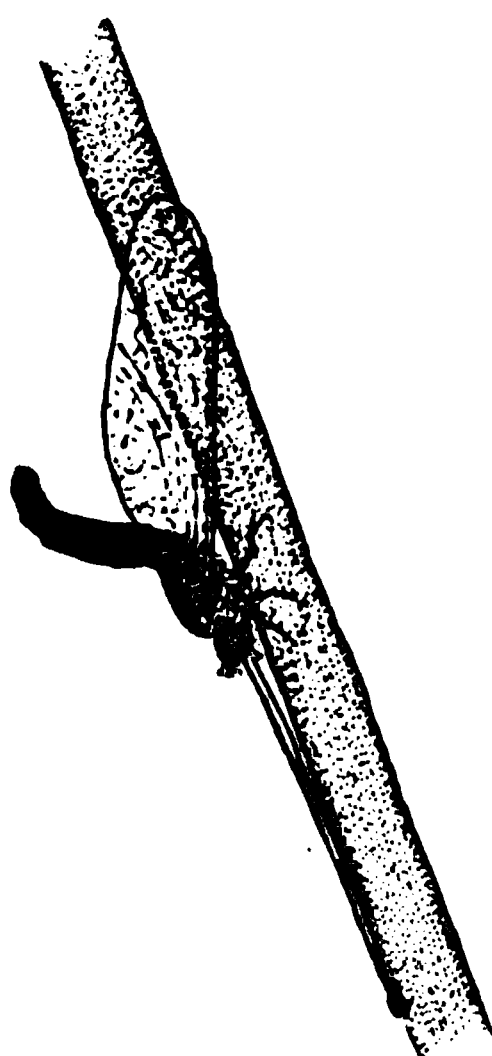


FIG. 2. — *Ulula hyalina*. Male life size, resting on a stalk of green sedge.

Guilding says the eggs (Fig. 3) are placed in double series of 64 to 75, near the end of a branch, and are fenced off by little rods, which he called "repagula" (Fig. 3), placed on end and arranged in circles around the branch below the eggs, thus preventing the approach of insects and the wandering abroad of young larvæ until they can climb over the repagula and have likewise acquired strength enough to resist ants and other insect enemies. I observed one of my specimens from Galveston deposit its eggs and repagula; but it was too weak

¹ Uebersicht der Myrmeleoniden-Larven, *Denkschriften der Kaiserlichen Akademie der Wissenschaften*, Bd. xlviii, Taf. VII. Wien, 1884.

from confinement to remain on the stalk on which it rested and fell to the ground, so that I could not tell how it would have arranged the eggs under normal circumstances. These repagula I find to be in all probability abortive eggs, since dissection shows that some of the tubules of the ovary produce eggs, and others repagula.

The eggs hatch after nine to ten days. The young larva remains quiet a day or two, after which it seeks the ground.

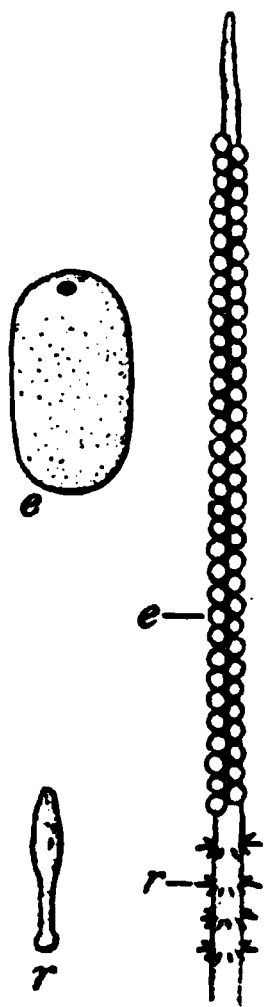


FIG. 3. — *Ulula hyalina*.
Egg and repagulum;
and a figure showing
the arrangement of the
eggs and repagula on a
stalk, according to
Guilding's description:
e, egg; r, repagulum.

The larva, while growing in size, always retains the form shown in Fig. 4, except that after hatching and after each moult the head is proportionally larger. It hides in some slight depression or under the edge of a stone, with its body covered with sand and its mandibles widely extended so as to touch the fringe of hairs on each side of the head. Its brown color simulates the surroundings. Its body is hidden by the covering of sand, and the head is somewhat concealed by its peculiar covering of hairs (Figs. 4, 5, 12), so that small insects may crawl, unawares, too near the extended mandibles. In this case the larva thrusts out its head and snaps the mandibles together, pinioning the victim on the curved points. It then proceeds to suck out the juices of its prey like an ant-lion. In the latter this is accomplished, according to Redtenbacher, by the expansion of the pharynx, the juices passing through the

duct formed by the mandible and maxilla fitting together (Fig. 6). The wound is kept open by the maxilla working like a piston in the groove of the mandible. The palpi at the same time move back and forth slightly. The labium and ligula are folded back into the mouth and adhere together so as to close the orifice in front. I have observed that the *Ulula* larva soon kills its victim, and at intervals opens its mandibles slightly, until one of them comes out, and then sticks it into a new place. This is continued until the skin is sucked dry,

when the larva throws it aside and assumes its characteristic attitude and awaits another victim. On being disturbed the larva crawls away and seeks some other retreat. It often changes its hiding place at night, probably on account of scarcity of food. It always walks forward, contrary to the habit of ant-lions. The larval life lasts about sixty-two days, during which time the larva moults twice. It moults a third time inside of the cocoon, when it changes to the pupa.

As the habits of the *Ulula* larva are somewhat peculiar, it becomes of interest to compare them with the habits of the not very remotely related ant-lions (*Myrmeleonidæ*). According to Redtenbacher, the *Myrmeleonidæ* (*Formicaleo*, *Acanthaclisis*, and perhaps *Palpares*) lie quietly during the day like *Ulula hyalina*, except that the body, instead of being simply covered with sand, is buried slightly beneath the surface. They can walk backward as well as forward. At night they wander about in search of prey. *Myrmecælurus* can walk forward as well as backward, but digs a pit like the ordinary ant-lion. These latter, however, never walk forward. The digging of a pit by the ant-lion may be but a step removed from the habit of *Palpares* in burying its body. The ant-lion also has the instinct of wandering at night in search of a more favorable situation when food is scarce.

When the *Ulula* larva is full-grown it seeks some hidden place at night in which to pupate. Having found such a place, it spins a web, covering it with sand and such other small objects as may be at hand. It then gets inside the web and begins spinning a cocoon. The next day it remains quiet, and at night continues the work. I had an opportunity of observing several stages of the process, as my specimen spun its cocoon against the side of a glass jar partly filled with sand. The side next

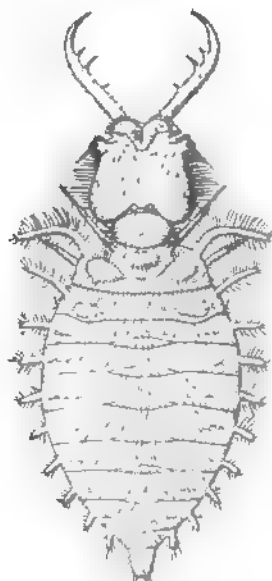
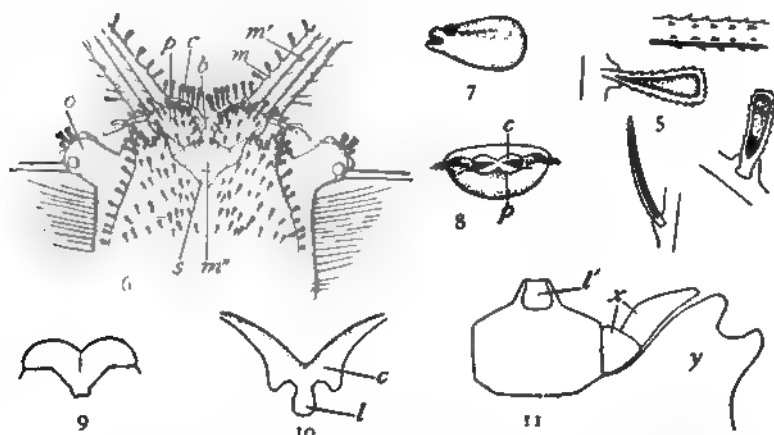


FIG. 4.—*Ulula hyalina*. Full-grown larva.

to the glass could be seen through until after the third night, so that it must have required more than three days to construct the cocoon.

The mandibles (Fig. 15, *m*) of the pupa are toothed on their inner edges for the purpose of enabling it to bite a hole in the cocoon when the metamorphosis has been completed. I did not have an opportunity to observe the escape of the imago.



FIGS. 5-11. — *Ulula hyalina*. Larva. FIG. 5, three small setae seen in optical section and a portion of a long one, surface view; FIG. 6, the head of a larval skin of the first moult, seen from below; FIG. 7, head with mandibles pulled out and setae scraped off, seen from the side; FIG. 8, the same, from the front. FIG. 9, clypeus seen from above; FIG. 10, distal portion of the clypeus, seen from below. FIG. 11, inner surface of the mentum and adjacent integument. *c*, clypeus; *l*, labrum; *m*, mandible; *m'*, maxilla; *s*, submentum; *m''*, mentum; *p*, labial palpus; *d*, point of contact of mentum and clypeus; *f*, ligula; *x*, lobe of the mentum; *y*, lobe of the gena; *e*, ocular peduncle.

Below I have added descriptions of the egg, repagulum, larva, pupa, and cocoon.

Egg and Repagulum.

Egg (Fig. 3, *e*). — Length $1\frac{1}{4}$ mm. Ovoid, about twice as long as broad, cream-colored. An elevated ring, the micropyle, on upper pole.

Repagulum (Fig. 3, *r*). — Length $1\frac{1}{2}$ mm. Slender, spindle-shaped, knobbed at base. Basal knob red; shaft brown.

The repagula are abortive eggs. Some tubules of the ovary bear eggs, others repagula.

According to Guiding the eggs are placed in a double alternating series of 64 to 75, near the extremity of the branch, and the repagula (barriers) are placed in circles around the branch, below the eggs (Fig. 3).

Larva (Fig. 4).

Length 13 mm. Head cordate, broad behind, tapering anteriorly, swollen beneath, thicker behind than in front, fuscous varied with black, covered with hair except on mid-ventral line, deeply emarginated on posterior border above, leaving an angular projection in center of emargination. Anterior border fringed with serrated hairs; lateral borders each with a very deep fringe. Ocular peduncles (Fig. 12, *o*) prominent, cylindroid, slightly flattened dorso-ventrally, each bearing seven simple eyes and thickly beset at the end with serrated hairs, two of which are very large and inclined backward. Eyes black, six on upper surface of ocular peduncle, five forming an incomplete circle around the sixth, one on under surface of ocular peduncle near the posterior outer margin. Antenna (Fig. 12, *a*) $1\frac{1}{2}$ times

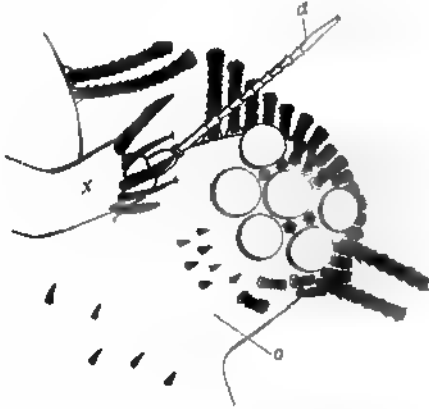


FIG. 12.—*Ulula hyalina*. Ocular peduncle of larva: *o*, ocular peduncle; *a*, antenna; *x*, lobe over antenna.

as long as ocular peduncle, basal segment very large and broad at base, second segment much smaller, third still smaller and proportionately much shorter, and followed by a piece not definitely segmented and still more slender; the succeeding nine segments are of nearly equal size, the terminal segment is as long as the three preceding and ends in a tuft of small hairs,

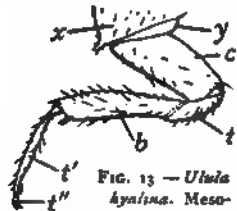


FIG. 13.—*Ulula hyalina*. Mesothoracic leg of larva: *x*, articular membrane; *c*, coxa; *t*, trochanter; *b*, femur; *t'*, tibia; *t''*, tarsus.

there is a lobe above the base of the antenna ending in a fringe of five hairs,—three stout serrated ones alternating with two slender smooth ones which terminate in stellate enlargements. Clypeus (Fig. 9) narrow behind, continued in front as a pair of semicircular lobes over bases of mandibles, middle of anterior border continued downward and reaching the mentum. Labrum (Fig. 10, *l*) small, infolded into the mouth. Mandible long, swollen at base, straight for three-fourths its length, then curving inward; curved portion free from hairs, inner edge set with three teeth, middle

one largest, posterior one smallest, space between anterior and middle one smaller than that between middle and posterior one, mandible grooved on ventral side. Maxilla swollen at base, lying in groove of mandible, with which it forms a duct leading to the mouth, serrated on inner edge near tip. Mentum (Fig. 6, *m''*) nearly square, anterior border produced in the middle so as to

reach the clypeus, lateral margins produced into a pair of triangular lobes (Fig. 11, *x*) inclined forward and devoid of hairs, each divided by a suture into two triangles; ligula infolded into mouth, adhering to the labrum; basal segment of labial palpus very large, flattened, fourth segment as long as second and third combined. On each side of the mentum arises a lobe of the gena (Fig. 11, *y*) which projects over the base of the mandible, deeply emarginated on anterior border. The hairs (Fig. 5) on the head, as well as on the body, are of peculiar structure: the base of a hair is constricted so as to close the cavity within, and the integument is raised around the constricted portion; the hairs are of two kinds, smooth and serrated; some of the serrated hairs are enlarged at the tip.

Thorax flat, much broader behind than in front, luteo-fuscous mottled with fuscous, thickly beset with hairs, all of which are fuscous or black;

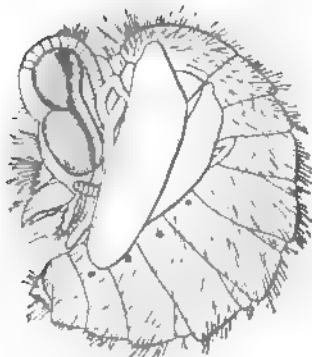


FIG. 14. — *Uta hyalina*. Pupa

prothorax freely articulated with mesothorax, much narrower than head, much broader than long; metathorax broader than head, bearing above three ellipsoid swellings, each of which has the margin depressed below the general level; one lies near the anterior border, the other two near the lateral borders and are connected by a deep groove; two pairs of lateral lobes, anterior pair short, conical, inclined forward, terminating in a few black setae which are packed together so as to appear like the end of the lobe, second pair very long, inclined forward at base, then bent slightly backward, fringed with large, serrated hairs; there is a pair of spiracles below near the bases of posterior pair of lobes; metathorax broader than mesothorax, a pair of ellipsoid swellings above, similar to those on mesothorax, a pair of lateral lobes, shorter than pair on mesothorax, inclined forward, fringed with large hairs; a pair of spiracles below, near bases of lateral lobes; legs luteo-fuscous with fuscous hair, anterior pair small, each succeeding pair larger, coxa very long (see Fig. 13),¹ trochanter small and almost rigidly attached to the femur, tibia slender, tarsus of a single small joint, unguis black, much curved.

Abdomen broad and thin, dorso-ventrally compressed, pointed behind, lateral margins very convex, luteo-fuscous mottled with fuscous, covered with hairs which vary from fuscous to black, flattened and wrinkled below,

¹ Redtenbacher (*loc. cit.*, Fig. 116) gives a different interpretation to the joints of the leg of the ant-lion. He calls coxa what I believe to be a much-developed articular membrane (Fig. 13, *y*), and what I have called the trochanter he regards as part of the femur. The articulation between the trochanter and femur is not well developed and allows very little movement, but it cannot be overlooked. I may add that my interpretation is based on comparison with the imago.

somewhat convex above, divided into nine segments; tergites, except last two, separated along middle, so as to disclose the articular membranes, — each tergite, except the last two, with a cross groove nearly reaching the lateral borders; each segment bears a pair of lateral lobes fringed with fuscous hairs; each segment also bears a pair of spiracles below, near the bases of the lateral lobes; last segment conical, truncated. There is a circle of black curved spines around anus.

Pupa (Fig. 14)

Length 12 mm., diameter in cocoon 6 mm., breadth of abdomen 4 mm. The pupa resembles the imago in general, but is much smaller and comparatively shorter.

Head short, and compressed against thorax; eyes fuscous, with a deep vertical fold at right angles to the sulcus; antennæ comparatively short,

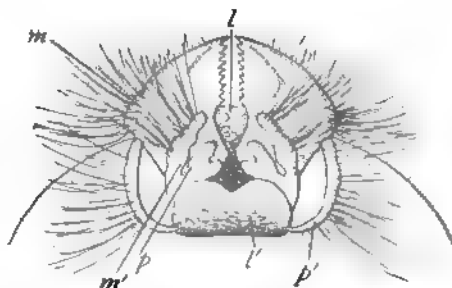


FIG. 15. — *Ulula hyalina*. Mouth of pupa from below: *l*, labrum; *m*, mandible, *p*, maxilla; *m'*, maxillary palpus; *l'*, labium, *p'*, labial palpus

curved backward over the head, not knobbed, white; face light yellow, clypeus not distinct from labrum; mandibles (Fig. 15) stout, armed each with nine to ten teeth; inner edge of mandible rufo-fuscous, teeth black; gular region white; maxilla bilobate, maxillary palpus of three segments; labium slightly bilobate; labial palpus slender, smaller at base than at tip; mouth parts and vertex clothed with fine white hair.

Thorax short, cream-colored varied with ferruginous; prothorax compressed against back of head; wing sacs small, white; legs slender, white.

Abdomen short, curved under so that the anus reaches the mouth, cream-colored, varied with ferruginous, covered with fine white hair, a narrow mid-dorsal groove; each segment except the last two with a pair of spiracles.

Cocoon spherical, diameter 7 mm., made of silk.

UNIVERSITY OF TEXAS, AUSTIN,
November 20, 1901.

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IN

ZOOLOGY

Vol. 6, No. 3, pp. 33-64, Pls. 1-6

July 15, 1909

CONTRIBUTIONS FROM THE LABORATORY

OF THE

MARINE BIOLOGICAL ASSOCIATION OF SAN DIEGO

XXV

**THE OPHIURANS OF THE SAN DIEGO
REGION**

BY

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CONTRIBUTIONS FROM THE LABORATORY
OF THE
MARINE BIOLOGICAL ASSOCIATION OF SAN DIEGO

XXV.

THE OPHIURANS OF THE SAN DIEGO
REGION

BY

J. F. McCLENDON.

My interest in Ophiurans lies chiefly in the experimental side, and the present work was begun in June, 1907, with the hope that a taxonomic and biological study of the San Diego species might greatly facilitate work in which it was required to know breeding seasons and habitats of the different species. Unfortunately I left San Diego September 1, 1907, before much data on breeding seasons had been collected. I believe that the height of the breeding season for the majority of species is in the spring, although individuals full of eggs that were apparently not ripe were found during the summer. Owing to the confused state in which the taxonomy of the Ophiurans has been left, I found it impossible to complete the work without much aid from Dr. H. L. Clark, for which my sincere thanks are due him. I am also indebted to Miss Rathburn and Dr. Austin Clark of the National Museum; to the museum itself for the loan of specimens, and to the John Crerar Library and the Universities of Michigan and California for literature.

Although most of the species react negatively to light and hide under rocks and in sea-weed, sponges, etc., some of them

appear to be protectively colored. The abundance and ease of collection of the littoral species leads me to believe that hybridization experiments might be worth trying and that the eggs might also be favorable for many other problems in experimental embryology. It would be of interest in this connection to know what species are viviparous.

In giving the diameter of the disc the largest specimen at hand was always chosen. Most of the species have been described and figured elsewhere, but the following descriptions and figures have the advantage to the student at San Diego in all being made from the region where his material will be procured. No dissections are described; for example, *only the exposed part* of the radial shield is described.

Types or representatives of all are in the University of California museum, except *Ophiocnida hispida*, which is in the United States National Museum. The figures were drawn by the author, excepting 24 and 25, which were drawn by Mr. G. T. Kline.

Class OPHIUROIDEA Norm. 1865.

Order 1. ZYGOPHIURAE Bell 1892.

Ophiuroidea with well-developed parts in the skeleton of the arm segments. The arms are not branched and cannot be rolled up toward the mouth.

Suborder A. BRACHYOPHIURAE E. Perr, 1891.

Arm spines short, parallel to the arm axis.

Family 1. OPHIODERMATIDAE Ljg. 1867.

With numerous mouth papillae, no tooth papillae, 2-4 genital openings.

Genus *Ophioderma*.

Ophiura Lm. 1801 (*partim*).

Disc granulated. Teeth, and numerous, equal, close-set mouth papillae. No tooth papillae. Spines smooth, flat, shorter than arm joints, numerous (7-13). Two tentacle scales; only the upper one covers the base of the last arm spine. A notch in the

disc over base of arm. Four genital openings in each interbrachial space; the first pair begins at the outside of the mouth shield.

***Ophioderma panamensis* Ltk.**

Fig. 1.

Additamenta ad Hist. Oph., II, p. 193.

Diameter of disc 25 mm. Length of arm about $3\frac{1}{2}$ -4 times diameter of disc. Disc granulated above and below so that only a minute portion of each radial shield is uncovered. Mouth papillae and teeth small. Mouth shields sub-triangular with the corners much rounded. Side mouth shields hidden by granules. Four genital openings in each interbrachial space. Tentacle scales flat and continuous with arm spines. Ventral arm plates about as broad as long, with corners rounded. Dorsal arm plates two to three times as broad as long with distal margin concave and corners rounded. Arm spines numerous, flattened, lying close to arm and shorter than arm joints. Color dark brown above, lighter below, the arms encircled by pale bands.

A common littoral species of the region. Panama to San Pedro and Catalina. San Diego M. B. L., La Jolla. Albatross stations 3588, 3589 and 3590, San Diego Bay. Smithsonian 5213, Catalina Id.; No. 1 San Diego (H. Hemphill). U. S. Nat. Mus. 12732 (b) Catalina Harbor 30-40 fathoms (W. H. Dall c 6 1874).

Family 2. OPHIOLEPIDIDAE Ljg. 1867.

Three to four mouth papillae, of which the innermost is seldom below the teeth. No tooth papillae. Notch in disc above base of arm. Two genital openings in each interbrachial space.

Genus ***Ophiomusium*** Ly. 1869.

Teeth. No tooth papillae. Mouth papillae fused in a continuous row so that their former outlines are indistinct. Disc covered by plates and radial shields, all of which are fused. Upper and under arm plates minute, side arm plates meeting above and below, swollen and fused with neighboring plates. No tentacle pores beyond the basal arm joints. Small arm spines on outer edge of arm plates. Two genital openings in each interbrachial space.

***Ophiomusium jolliensis* sp. nov.**

Figs. 2, 3.

Diameter of disc 12 mm. Length of arm $2\frac{1}{2}$ times diameter of disc. Disc covered with irregular plates, variable in size and shape, the marginal interbrachial ones very large. Five or six pairs of mouth papillae to each jaw. Teeth very acute. Mouth shields lanceolate, the point directed inward and sometimes separating the side mouth shields. Two genital openings in each interbrachial space. One tentacle scale small and flat. Only three pairs of tentacle pores on arm (3 basal segments). The three proximal ventral arm plates are pentagonal and bear tentacular openings. The remaining ones are sub-triangular or quadrangular with the inner margins convex. Dorsal arm plates lanceolate with the point directed proximally. Two or three short blunt arm spines. Color of preserved specimens cream or light grey.

San Diego M. B. L.: 1026 (Station 6, San Clemente), 215 fathoms. 972, four miles W.N.W. of La Jolla, 125 fathoms. 1038 (Station 13), 308 fathoms. 974, three-fourths mile N.N.W. of La Jolla, 85 fathoms. 1027 (Station 8, San Clemente), 330 fathoms. 1534. 1541.

***Ophiomusium lymani* W. Thos.**

Figs. 4, 5.

"Depths of the Sea," p. 172, figs. 32, 33.

Diameter of disc 30 mm. Length of arm about 4-5 times diameter of disc. Disc covered with irregular polygonal fused scales. Radial shields very large and tuberculated, as is also the large marginal scale between them. Five to seven rectangular fused mouth papillae on each side. The outer ones may so fuse as to reduce the number. Mouth shields narrow and pointed at inner end. Side mouth shields large, meeting within, the outer ends much broader than the inner. Genital openings usually reaching only to the 2nd or 3rd arm joint, the inner ends curved. Tentacle scales small and flat. Tentacle pores in 1st and 2nd arm joints only. Under arm plates in 1st and 2nd arm joints only. Side arm plates meeting above and below. Upper arm

plates small and diamond shaped. Six to eight rudimentary arm spines. Color of preserved specimens grey.

This is a deep-sea cosmopolitan species. San Diego M. B. L., 1072 (La Jolla Sta. III, 600 fathoms).

Genus **Ophiura** Lm. 1816.

Ophiolepis M. & T. 1842 (*partim*).

Ophioglypha Ly. 1865.

Disc covered with plates or scales which are often swollen. Radial shields naked and swollen. Teeth. No tooth papillae. Mouth papillae long within, but small and short near outer end of the mouth slit and partly hidden by the scales of the mouth tentacles. Arm spines short and smooth, rarely exceeding the length of a joint. Tentacle scales numerous, the innermost pair of tentacle pores shaped like slits surrounded by numerous tentacle scales and opening diagonally into the mouth slit. In the disc, over the base of the arm, is a notch usually edged with papillae. Two genital openings starting from the sides of the mouth shield.

Ophiura lütkeni Ly.

Figs. 32, 33.

Proc. Bost. Soc. Nat. Hist., VII, p. 197, 1860.

Diameter of disc 8 mm. Length of arm about $5\frac{1}{2}$ times diameter of disc. Disc flattened, covered above with slightly swollen scales and radial shields. Each larger scale surrounded by smaller ones. Radial shields small, ovoid, pointed within. A notch above the base of arm. Interbrachial spaces below covered with slightly swollen scales. Eight pairs of mouth papillae, the inner ones spine-like and separated, the outer four broad and close together. Teeth. No tooth papillae. Mouth shields pentagonal, the inner angle acute, the others rounded, lateral edges emarginate. Side mouth shields long, narrow and meeting within. Genital openings beginning at the sides of the mouth shield. First and sometimes second tentacular openings, with two or three flat scales, succeeding four or five each with one flat scale and one minute spine-like scale, the remainder each with one flat scale. Ventral arm plates twice as broad as long, small

and well separated, proximal margin angular, distal margin convex. Side arm plates meeting below. Dorsal arm plates narrowed and truncated proximally, the distal margin produced into an obtuse rounded angle. Three tapering arm spines on the proximal joint, the dorsal one about as long as the arm joint. On the remaining joints the dorsal spine is a little shorter than the arm joint. The other two spines are shorter than the dorsal spine. Color of preserved specimen grey above and white below.

San Diego to Puget Sound, 22-600 fathoms. San Diego, M. B. L.: 1025, San Clemente, 60 fathoms. 1072, La Jolla Station III, 600 fathoms. 1112. 1288, $2\frac{1}{4}$ miles S.W. by S. of La Jolla, 50 fathoms. 1497. 1501. XII-2, June 5, '01, 30 fathoms. XIII-1, June 6, '01. XIV, 2, San Pedro, 100 fathoms. XIX, 2-5, San Pedro, 77 fathoms. XX, 1, San Pedro 77, fathoms. XXI-2, June 20, '01, 43 fathoms. XXVI-a. XXXI-a, near Potts Valley, San Pedro. L-1 and 2, off Pt. Loma, 25 fathoms. LV-1 and 2, San Diego, 25 fathoms. LXX-5, La Jolla, submerged valley, 100 fathoms. LXXII-1, off Pt. Loma, 30 fathoms. Smithsonian: 5214 Catalina.

***Ophiura kofoidi* sp. nov.**

Figs. 8, 9.

Diameter of disc $7\frac{1}{2}$ mm. Length of arm about four times diameter of disc. Disc thick but flat, pentagonal, covered with imbricated scales and radial shields. A large central scale. A notch over base of arm bordered by two rows of spines, one row being continued along genital opening. Five to six pairs of mouth papillae, the innermost sometimes spine-like. Teeth. No tooth papillae. Mouth shields large, pear shaped, the stem of the pear being a sharp angle pointing inward. Side mouth shields, narrow, meeting within. Genital openings starting between mouth shield and side mouth shields. First tentacle pore opening diagonally into mouth angle and surrounded by nine scales, second (in 1st arm joint) surrounded by six scales, the remainder each with two spine-like scales. Ventral arm plates short and well separated, convex distally, produced to a point proximally. Side arm plates meeting below. Dorsal arm plates broader than long in proximal part of arm, distal margin convex, narrowed proximally. Arm spines numerous, slender, acute, slightly curved, on the proximal joints the dorsal spines are $2\frac{1}{2}$ times length of arm joint, the ventral spines a little longer than

arm joint. Color of preserved specimen grey above, lighter below.

San Diego M. B. L.: 991, $1\frac{3}{4}$ miles N.W.N. of La Jolla, 80 fathoms.

Genus *Ophioplocus* Ly. 1861.

Disc closely and finely scaled above and below. Genital scales hidden. Teeth. No tooth papillae. Numerous even, close set mouth papillae. Side mouth shields wide and nearly or quite meeting within. Three short arm spines. Upper arm plates, near tip of arm, divided on midline into halves, which at base of arm are removed to outer lower corner of joint on each side and separated by a number of supplementary pieces. Two short genital openings in each interbrachial space, extending only half way to the margin of the disc and beginning outside the side mouth shields.

***Ophioplocus esmarki* Ly.**

Figs. 6, 7.

Bull. Mus. Comp. Zool., III, pt. 10, p. 227, pl. V.

Diameter of disc, 30 mm. Length of arm $2\frac{4}{5}$ times diameter of disc. Disc and arms flattened. Radial shields nearly covered by scales. Scales irregular and swollen, giving the disc a pebbled appearance, the larger ones, above, being usually surrounded by smaller ones. The scales on the ventral side smaller than those on the dorsal and more uniform in size. Ten to twelve mouth papillae. Mouth shields sub-triangular, aboral margin rounded. Genital openings, two in each interbrachial space, beginning near the mouth shields, the edges granulated, and the granulation extending out to the margin of the disc. Tentacle scales usually 4-5 in number surrounding each tentacle pore. Ventral arm plates six-sided, narrowed behind, distal margin straight or slightly incurved. Dorsal arm plates broken into a large number of irregular pieces. Arm spines three in number, about two-thirds the length of the arm joints. Color of preserved specimens light or dark brown or bluish grey.

San Diego to Pacific Grove. Shore to 40 fathoms. San Diego M. B. L.: 1023, San Clemente, San Pedro, Sta. 5, 15 fathoms. Albatross: March 15, 1891, off Point Loma. U. S. N. M.: 12732 (a), Catalina Harbor, W. H. Dall

(c 6), 30-40 fathoms, 1874. No. 2 (Henry Hemphill), San Diego. Smithsonian: 12644, W. H. Dall (c 7), Catalina Harbor, 40 fathoms. 4290, Catalina.

Genus **Ophiocten** Ltk. 1854.

Disc thick and circular, partly covered by plates and radial shields between which are sometimes fine, close-set grains or small scales. Genital opening bordered by a row of papillae that often extends upward along the edge of the disc, over the arm base. Side arm plates come together below but not above. The broad mouth tentacle is enclosed between the first ventral arm plate and the outer edge of the side arm plate. Teeth. Mouth papillae. No tooth papillae. Two genital openings beginning at the sides of the mouth shield.

Ophiocten pacificum L. & M.

Figs. 14, 15.

Mem. Mus. Comp. Zool., XXIII, No. 2, 1899.

Diameter of disc 12 mm. Length of arm a little more than three times diameter of disc. Disc covered with separated plates and radial shields and the areas between them covered with imbricated scales. Radial shields narrowed within. Five to six pairs of mouth papillae in each jaw, the inner one spinous. Teeth. No tooth papillae. Mouth shield pentagonal, inner angle sharp, remaining ones rounded. Side mouth shields meeting within, narrowed without. Genital openings begin at sides of mouth shield, bordered with papillae that are continued up over arm. First pair of tentacle pores between basal arm plate and side mouth shields, each with two broad scales. Second pair of tentacle pores each with a bilobed scale. The remainder, each with one flat and one minute spinous tentacle scale. Ventral arm plates small and well separated. The first one trapezoidal, narrowed proximally. Side arm plates meeting below. Dorsal arm plates broader than long, narrowed proximally and convex distally; the proximal plates bearing minute spinous papillae on their distal margin. Three slender, slightly curved arm spines, the dorsal spine longer than two arm joints, the ventral spine a little longer than one arm joint.

From Galapagos Ids. and Gulf of Panama to San Diego; down to 1573 fathoms.

Sub-order B. **Nectophiurae** E. Perr, 1891.

Spines on arms perpendicular to arm axis.

Section 1. **OLIGODONTIDA** Ludwig & Hamann, 1901.

Tooth papillae few or wanting.

Family 3. **AMPHIURIDAE** Ljg. 1867.

One to five mouth papillae, the innermost often infradental. Arms arising from the ventral side. Two genital openings.

Genus **Ophiopholis** Müll. & Tr. 1842.

Disc more or less covered with grains or little spines. No tooth papillae. Mouth papillae on the sides of the mouth frames. Arm spines short, flat and stout. Upper arm plates surrounded by a rim of supplementary pieces. The lowest spine of the distal arm joints is a hook. General structure coarse and stout. Two genital openings beginning outside the mouth shields.

Ophiopholis bakeri sp. nov.

Figs. 26, 27.

Diameter of disc $6\frac{1}{2}$ mm. Length of arm four to five times diameter of disc. Disc (with exception of mouth parts) completely covered with short, thorny spines. Teeth sometimes divided in the middle and having rudiments of papillae. Five pairs of slender papillae. Mouth shields diamond-shaped, corners rounded, nearly twice as long as broad. One long, flat and narrow tentacle scale. Ventral arm plates octagonal, distal, lateral and proximo-lateral edges concave. Dorsal arm plates circular, each surrounded by a row of small, round supplementary pieces. Six arm spines in proximal portion of arm, decreasing to four in distal portion, minutely thorny. First spine (above) of variable length, second (longest) a little longer than arm joint, the remainder decreasing in length downward. Color of dried specimen pink or red.

San Diego M. B. L.: 1025, San Clemente, Station 5, 60 fathoms. 1026, San Clemente, Station 6, 215 fathoms. 1047, 4 miles N.W. by N. of La Jolla, 100 fathoms. 1155, $2\frac{1}{2}$ miles W.N.W. of La Jolla, 70 fathoms. XXVI-a. LXX-5, Soledad, submerged valley, Aug. 23, 1901, 100 fathoms. 1501. 1532. 1534. 1537. 1549.

Genus **Ophiactis** Ltk. 1856.

Disc round and stout and covered with radial shields and imbricated scales, the latter usually bearing small spines. Teeth. No tooth papillae. Mouth angle short and narrow, with few (2-4) small mouth papillae. Arms somewhat depressed, in length 4-7 times the diameter of the disc. Arm spines short and smooth. Two genital openings, beginning outside the mouth shields.

Ophiactis arenosa Ltk.

Fig. 16a. *Ophiactis arenosa*, Ltk., partial view.

Fig. 16b. Partial ventral view of same.

Lütken, C. F. "Bidrag til Kundskab om Slægtstjerne, III,"
Vidensk. Meddel. naturhist. Foren i Kjobenh. 1856.

Diameter of disc 6 mm. Length of arm about three and one-third times diameter of disc. Disc covered with radial shields and imbricated scales, the latter in interbrachial spaces and on edge of disc bear each a small spine on its outer edge. Teeth. No tooth papillae. One or two pairs of thin mouth papillae in each jaw. Mouth shield quadrilateral, inner angle sharp, the other angles rounded, outer edges convex. Side mouth shields meeting to form a continuous ring round the mouth. Genital openings beginning at mouth shield. One flat tentacle scale. Under arm plates about as broad as long. Upper arm plates broader than long, narrowed proximally. Five arm spines, upper one less than one arm joint, middle three each about one and one-half arm joints and lower one about one arm joint in length. Color of dried specimen cream with fuscous mottlings.

West coast of Central America. San Diego Bay near National City, in sponges, June, 1908, 15 specimens.

Genus **Amphiura** Forbes 1842.

Disc small and delicate, covered with overlapping scales and naked radial shields. Teeth. No tooth papillae. Mouth angle small and narrow with few (usually 4-6, seldom 8-10) small mouth papillae. Arm long, slender, even and more or less flattened. Arm spines short and regular. Two genital openings in each interbrachial space.

Sub-genus **Amphiura** Verrill 1899.

One apical or sub-apical mouth papilla. One (rarely 2) small, distal papilla (oral tentacle scale). Middle of jaw edge without papillae. Mouth slits gaping. Four to seven or more (rarely 3) arm spines. Radial shields divergent.

Amphiura verrilli sp. nov.

Figs. 10, 11.

Diameter of disc 8 mm. Length of arm five times diameter of disc. Disc above and below covered with imbricated scales. Radial shields naked, separated, and about twice as long as broad. Three pairs of mouth papillae. Teeth. No tooth papillae. Mouth shield diamond shaped, the edges next to the genital openings concave, corners rounded. Side mouth shields narrowed and meeting within, broad without. Genital openings beginning at mouth shields. One flat tentacle scale. Ventral arm plates concave on distal margin, narrowed proximally. Side arm plates meeting above, meeting below except at base of arm. Dorsal arm plates elliptical, about twice as broad as long. Four stout tapering arm spines, a little longer than arm joints. Color of dried specimen light grey.

San Diego, M. B. L.: San Diego, 100 fathoms. XXXI-a, near Potts Valley, San Pedro.

Sub-genus **Amphipholis** Ljg. 1867-71.

Two small lateral and one broad operculiform distal mouth papillae, forming a continuous series along the entire jaw and capable of nearly or quite closing the mouth slits. Radial shields in close contact. Disc covered with scales (usually naked).

Amphipholis pugetana Ly.

Figs. 12, 13.

Proc. Bos. Soc. Nat. Hist., VII, p. 193, 1868.

Diameter of disc $3\frac{1}{2}$ mm. Length of arm about five times diameter of disc. Disc covered with imbricated scales, the dorsal ones longer and meeting those on the edge in a distinct line. Radial shields naked, about twice as long as broad, meeting along their whole length. Three pairs of mouth papillae, the outer

ones very broad. Teeth. No tooth papillae. Mouth shield small, quadrangular, the inner angle the most acute. Side mouth shields narrowed within, where they meet. Genital openings beginning at mouth shield. Two flat tentacle scales. Ventral arm plates pointed behind (where the side arm plates meet for a very short distance), about twice as broad as long, distal edge slightly concave. Dorsal arm plates sub-triangular, distal margin convex, corners rounded. Disc light yellowish brown, lighter below, radial shields darker, shading into a sooty white at their outer ends. Mouth and ambulacral regions white. Arms at sides and above and arm spines a sooty white.

San Diego to Puget Sound. San Diego M. B. L.: 1026, San Clemente, Station 6, 213 fathoms. 1038, Station 13, 308 fathoms. 1166, N.E. from Long Beach (La Jolla) to 1st canyon, 30 fathoms. XLIII-3, San Diego, channel west of middle ground, Ballast Point, 5 fathoms. LXII-1, July 20, 1901, 18 fathoms. Shelters Cove, June 27, 1894. Wilson's Cove, June 11, 1896. Albatross: San Diego, March, 1898. Smithsonian 14182, Catalina, W. H. Dall. Smithsonian 12710, Catalina Harbor (c 6), 1873, 30-40 fathoms, sandy mud.

***Amphipholis puntarenæ* Ltk.**

Figs. 20, 21.

Diameter of disc $7\frac{1}{2}$ mm. Length of arm about 7 times diameter of disc. Disc covered above by large imbricated scales and naked radial shields that meet along outer third. Inter-brachial space granulated. Three pairs of mouth papillae, outer one very broad, next narrower and inner one small and partly infradental. Mouth shield quadrangular, corners rounded, inner angle most acute. Side mouth shields, broad, meeting within, sub-triangular, corners rounded. Genital openings beginning at mouth shield. Two flat tentacle scales, the distal one larger. Ventral arm plates quadrangular, distal corners rounded, broader than long. There is a minute plate separating the outer ends of the side mouth shields that may be a vestige of the first ventral arm plate. Dorsal arm plates ellipsoidal, about twice as broad as long. Three stout sub-equal arm spines about the length of an arm joint. Color of preserved specimen grey.

San Diego M. B. L.: 1289-c, 4 miles S.W. by S. of La Jolla, 50 fathoms, 3 specimens. LXX-5, La Jolla, submerged valley, July 23, 1901, 100 fathoms, one specimen. Smithsonian: 12806, San Diego, H. Hemphill, 10 fathoms, 1 specimen.

Sub-genus **Amphiodia** Verrill 1899.

Three (rarely 4) small, subequal mouth papillae, none of them operculiform. They form a regular series attached mostly to the side jaw plate. No distinct oral tentacle scales. Three (rarely 4) arm spines. Radial shields often more or less joined.

Amphiodia barbarae Ly.

Figs. 22, 23.

Ill. Cat. Mus. Comp. Zoo. Harvard, VIII, pt. 2, p. 17, pl. III.

Diameter of disc 4 mm. Length of arm about 12 times diameter of disc. Disc covered with small imbricated scales. Radial shields naked, meeting along outer two-thirds of length, twice as long as broad. Three pairs of thick mouth papillae. Teeth. No tooth papillae. Mouth shield quadrangular, outer angle more obtuse, corners rounded. Side mouth shields very slender, meeting within, outer ends knobbed. Genital pores beginning at mouth shield. Two very short, flat tentacle scales. Ventral arm plates heart-shaped, with apex proximally. Side arm plates meeting below. Dorsal arm plates elliptical, about twice as broad as long. Three tapering arm spines, subequal, a little shorter than an arm joint. Color of dried specimen yellowish, disc sometimes greenish.

San Diego to Santa Barbara. San Diego M. B. L.: San Pedro, Station 1-1, May 22, 1901. San Pedro, Station (8), June 25, 1895. XXXI-a, June, 1901. XXXIV-a, up Little Harbor Valley, June 28, 1901. XXXVI-c, June, 1901. LXXIII-1, off Pt. Loma, July 25, 1901, 120 fathoms. 974, 1½ miles N.W. by N. of La Jolla, 85-100 fathoms. 1112.

Specimens from San Pedro (8) and XXXVI have more rectangular dorsal arm plates and some have more pointed mouth papillae. In young specimens from XXXIV-a the scales on edge of disc project as trifid spines.

Genus **Ophiocnida** Ly. 1865.

Disc small and delicate, furnished with naked radial shields and naked overlapping scales, the latter beset with small thorns or grains. Teeth. No tooth papillae. Mouth angles short and small, bearing a few (4-6) little mouth papillae. Arms long, slender, even, more or less flattened. Arm spines short and regular. Two genital openings in each interbrachial space.

***Ophiocnida hispida* Le Conte.**

Figs. 34, 35.

Proc. Acad. N. Sc. Phila., V, p. 318, 1851.

Diameter of disc 7 mm. Length of arm about $8\frac{1}{2}$ times diameter of disc. Disc covered with small imbricated scales bearing pointed spines (not shown well in figure). Radial shields close together but not touching, 2-3 times as long as broad. Teeth. No tooth papillae. Three pairs of rounded mouth papillae, inner pair appearing like a split tooth, outer pair broader than the rest. Side mouth shields broad without, narrow within, meeting or nearly meeting within. Mouth shields varying in size, diamond shaped, elongate radially. Genital pores beginning at mouth shield. Two flat tentacle scales. Ventral arm plates rectangular, about $1\frac{2}{3}$ times as broad as long, with corners rounded. Dorsal arm plates elliptical, about three times as broad as long. Three arm spines, blunt, flattened, about $1\frac{1}{3}$ times as long as arm joint, subequal except on distal end of arm where the dorsal spine is smallest. Color in alcohol, pale yellow, with small fuscous spots on disc and a larger fuscous spot near middle of each radial shield. Arms brown, interrupted with pale yellow with a median discontinuous fuscous line above. Arm spines pale, tinged with brown. Tentacle scales pale. Mouth parts pale, tinged with fuscous.

From west coast of Central America to Catalina. Smithsonian: 4100, Catalina, W. H. Dall (c 32).

***Ophiocnida amphacantha* sp. nov.**

Figs. 24, 25.

Diameter of disc $7\frac{1}{2}$ mm. Length of arm about 10 times diameter of disc. Disc covered with imbricated scales, some of which bear large, pointed spines. Radial shields narrow and touching without. Four pairs of mouth papillae on each jaw, besides a pair over the mouth tentacles. Teeth. No tooth papillae. Mouth shield pointed within, outer edge short and straight, edges next the side mouth shields convex, edges next the genital openings concave. Side mouth shields narrowed and touching within. Genital openings beginning very near the

mouth shield. Two very short, flat tentacle scales. Ventral arm plates rectangular with the corners cut off and the distal margin concave, longer than broad. Dorsal arm plates oval, and broader than long except at base of arm. Five to six stout, tapering arm spines, almost as long as arm joint. Color (preserved): arm yellowish, disc greenish.

San Diego M. B. L.: XXI-5, June 21, 1901, about 150 fathoms; XXXIV-a, up Little Harbor Valley, June 28, 1901. LXXIII-1, off Point Loma, July 25, 1901, 120 fathoms.

Genus *Ophionereis* Ltk. 1859.

Disc covered with fine, overlapping scales. Radial shields nearly hidden by scales. Large, oblong teeth. Mouth angle small and short and bearing 9-10 small, close-set papillae. A few (3-5) short, smooth arm spines. One large tentacle scale. Each upper arm plate has a supplementary piece on either side. Two genital openings beginning outside the mouth shield.

Ophionereis annulata Le Conte.

Figs. 36, 37.

Proc. Acad. N. Sc. Phila., V, p. 317, 1851.

Diameter of disc 13 mm. Length of arm about six times diameter of disc. Disc puffed in interbrachial spaces and covered with minute imbricated scales which nearly hide the radial shields. Mouth papillae, 5 pairs on each jaw and one pair in the mouth angle. Teeth. No tooth papillae. Mouth shields hexagonal. Side mouth shields narrowed and meeting within. Genital openings beginning at the mouth shield. One flat oval tentacle scale. Ventral arm plates square, corners rounded, distal margin concave. Dorsal arm plates rounded and narrowed distally. Three stout, flattened arm spines tapering distally, about $1\frac{1}{2}$ times the length of arm joint, dorsal spine shorter than the others.

West coast of Central America to San Pedro, shore to 35 fathoms. San Diego M. B. L.: VIII, San Pedro Harbor, May 29, 1901. XLIV-1, Quarantine Station to Beacon $3\frac{1}{2}$, San Diego, 5 fathoms. LXVI-1, Beacon 5 to $3\frac{1}{2}$, along north side of channel, San Diego, 8 fathoms. Whites Point, San Pedro, June 4, 1901. U. S. N. M.: No. 5, Henry Hemphill, San Diego. U. S. F. C.: San Diego. Albatross: Sta. 3620, San Diego Bay.

Family OPHIACANTHIDAE E. Perr. 1891.

Side arm plates well developed, usually meeting above and below. Numerous long spines. Mouth papillae usually numerous, forming a continuous row. First ventral arm plate usually concave within and bearing two papillae. Generally one median tooth papilla, sometimes several or a large cluster.

Genus *Ophiacantha* M. T. 1842.

Disc swollen and covered with a thin skin that obscures the underlying coat of fine imbricated scales, sometimes covers the radial shields and bears spines, thorns or rough grains. No tooth papillae. Mouth angle large and bearing 7-16 papillae. Four to eleven usually rough or thorny arm spines. Side arm plates nearly or quite meeting above and below. Two genital openings.

Ophiacantha normani Ly.

Figs. 18, 19.

Bull. Mus. Comp. Zoo. Harvard, VI, No. 2, p. 58, pl. XI.

Diameter of disc 27 mm. Length of arm about 6 times diameter of disc. Disc covered with imbricated scales bearing small sharp spines and partly covering the radial shields. One median and 4 pairs of mouth papillae on each jaw and one minute pair in corner of mouth. Mouth shield compressed radially, extended aborally into a blunt process. Side mouth shields narrow, meeting within. Genital pores beginning at mouth shield. One small sharp tentacle scale. Ventral arm plates ellipsoidal. Side arm plates meeting below. Dorsal arm plates triangular and bearing minute papillae on their convex distal margins. Four slender sharp arm spines, the upper one sometimes as long as three arm joints, the others decreasing in length downward. Color (preserved) muddy white.

Eastern and Western Pacific. San Diego M. B. L.: 1072, La Jolla Station, 600 fathoms.

SECTION 2. POLYDONTIDA, Ludwig & Hamann, 1901.

Tooth papillae numerous.

Family OPHIOCOMIDAE Ljg. 1867.

Mouth papillae. Teeth. Arms arise from ventral side of disc. Moderate or long spines perpendicular to arm. Disc, at least above, covered with granules on a soft skin, seldom with scales and naked radial shields. Mouth shields small or medium size, not extending into interbrachial space. Two genital pores.

Genus *Ophiopteris*, E. A. Smith, 1877.

Disc granulated. Radial shields covered. Mouth angle small and short and with small mouth papillae. Tooth papillae very numerous and arranged in a close, vertical lump. Four teeth, Arm spines smooth and solid, the upper one having one or two supplementary scale-like spines applied to its base. One tentacle scale. Two genital openings beginning outside the mouth shield.

Ophiopteris papillosa Ly.

Figs. 28, 29.

Ill. Cat. Mus. Comp. Zoo. Harvard, VIII, pt. 2, p. 11, 1875.

Diameter of disc 11 mm. Length of arm about $3\frac{1}{2}$ times diameter of disc. Disc completely covered above with stout cylindrical stumps. Interbrachial space covered with more slender stumps. Four to six pairs of small mouth papillae on each jaw and one small pair in the corner of the mouth (tentacle scales). Tooth papillae in four or five vertical rows, those in the middle being irregular. Mouth shields sub-triangular, with rounded corners. Side mouth shields narrow, meeting within. Genital openings beginning at mouth shield. One flat tentacle scale. First ventral arm plate small and sub-triangular with outer corners cut off and slightly broader than long. Dorsal arm plates hexagonal, about $1\frac{1}{2}$ times as broad as long. Five flat, blunt arm spines, the first (dorsal) and sometimes the second are reduced to scales, the third and sometimes the fourth about as long as three arm joints, the fifth usually shorter than two arm joints. The spines are very finely serrated toward their tips. Color, brown.

Lower California to San Diego. San Diego M. B. L.: XVIII-1, June 12, 1901, 30 fathoms. LI, off Pt. Loma, 25 fathoms. LXII-1, July 20, 1901, 17 fathoms. LXVIII-1, July 22, 1901, 30 fathoms. Albatross: March, 1898, San Diego.

Genus **Ophiothrix**, M. T. 1842.

Disc set with thorny grains or thorny spines. Radial shields large, triangular, swollen. Numerous crowded tooth papillae forming a vertical oval. Teeth. No mouth papillae. Five to ten arm spines often three times as long as the arm joints. Usually a small, spine-like tentacle scale. The base of the jaw pierced with a hole. Interbrachial spaces swollen. Two genital openings, beginning outside the mouth shield. Outer arm joints with hooks.

Ophiothrix spiculata Le Conte.

Figs. 38, 39.

Proc. Acad. N. Sc. Phila., V, p. 318, 1851.

Diameter of disc 15 mm. Length of arm about $5\frac{1}{2}$ times diameter of disc. Disc including radial shields covered with thorny spines. Radial shields nearly touching above base of arm. Tooth papillae in a linear vertical oval with a vertical row in the middle. No mouth papillae. Mouth shields compressed radially. Side mouth shields sub-triangular. Genital openings beginning at mouth shield. One small tentacle scale. Vertical arm plates a little broader than long. Dorsal arm plates pentagonal, $1\frac{1}{2}$ times as long as broad. Seven long, serrated arm spines, the 2nd or 3rd being longest and about $4\frac{1}{2}$ times the length of arm joint. Color, greenish brown, sometimes yellowish. Arms interrupted with orange bands. A cluster of orange spots near base of each arm on upper side of disc and internal to these the disc is speckled with orange (in one case the central area was white while the rest of the animal was colored normally). Mouth region whitish.

West coast of Central America to Pacific Grove. Shore to 100 fathoms. Very abundant. San Diego M. B. L., stations: VI, San Pedro, outer harbor, 11 fathoms. VIII, San Pedro, harbor, May 29, 1901. XIV-2, San Pedro, about 100 fathoms. XVI-2, 3, San Pedro, 9 and 13 fathoms. XVIII-1, June 12, 1901, 30 fathoms. XIX-5, about 77 fathoms. XXI-2,

June 20, 1901, 43 fathoms. XXIII-2, June 22, 1901. XXVII-b. XXVIII-a, off Isthmus Harbor, between White Rock and Fish Harbor, 12-30 fathoms. XXX, Potts Valley, San Pedro, 20 fathoms. XXXV-a, June 29, 1901, 30 fathoms. XXXVI-c. XXXVIII-a, 25 fathoms. XLVI-2, 56 fathoms. L-1 and 2, off Pt. Loma, 25 fathoms. LXII-1, 17 fathoms. LXVIII-1, San Diego Harbor. San Pedro, Sta. 3, June 21, 1895. San Pedro Sta. 15, June 29, 1895. Pacific Grove. On kelp roots from Portuguese Bend, June 1, 1901. San Clemente. Avalon, 1893. San Pedro Harbor, June 18, 1901.

Smithsonian: 7474, San Diego Bay, Rosa Smith. 12714, Catalina Harbor, W. H. Dall, January, 1874 (c 6), 40 fathoms. 12643, Catalina Harbor, W. H. Dall, January, 1874 (c 7), 40 fathoms. 12722, San Diego, Rosa Smith, ac. No. 14099. 12734, Catalina Harbor (c 32), U. S. N. M., 40-60 fathoms.

Albatross stations: 3576. 3620, San Diego Bay.

Ophiothrix rudis Ly.

Figs. 30, 31.

Bull. Mus. Comp. Zool. Harvard, III, pt. 10, p. 239, pl. III, 1874.

Diameter of disc 11 mm. Length of arm about five times diameter of disc. Disc covered with cylindrical spines, which bear microscopic thorns. Radial shields bare. Almost touching without. Numerous tooth papillae. No mouth papillae. Mouth shields quadrilateral, the inner angle obtuse, outer angle right and lateral angles acute. Side mouth shields narrowed and separated within. Genital openings beginning outside mouth shield. Tentacle scale similar to a small arm spine. Ventral arm plates quadrangular with corners cut off. Dorsal arm plates fan shaped. Five to six minutely serrated arm spines (including tentacle scales), the first (dorsal) or second being the longest and about $3\frac{1}{2}$ times length of arm joint, the rest decreasing in length downward. The general moss green color and character of the spines makes the animal resemble seaweed. Disc above green, often with reddish or bluish tinge. Radial shields sometimes with a few red or orange spots. Arm green (often yellowish) above with narrow fuscous and broad red cross bands; almost white below with broad spines usually green. Mouth region pale. Females with ripe eggs in summer.

Along shore, La Jolla. Salinas Cove, June 5, 1901. San Diego Channel, June 18, 1901. Smithsonian: San Diego. (Henry Hemphill), No. 4. 7471, San Diego, C. R. Orcutt (U. S. N. M., ac. No. 14530). Albatross: March 15, 1890, Pt. Loma.

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(For complete literature lists refer to Lyman, Th., "Report on the Ophiuroidea dredged by H. M. S. *Challenger*, during the years 1873-76." The Voyage of H. M. S. *Challenger*, Zool., Vol. 5, 1882, and Lütken and Mortensen, "Reports on an exploration off the west coasts of Mexico, Central and South America, and off the Galapagos Islands, in charge of Alexander Agassiz, by the U. S. Fish Commission Steamer *Albatross*, during 1901, Lieut. Comm. Z. L. Tanner commanding." The list below is merely of certain papers published too late to be included in either of the reports mentioned.)

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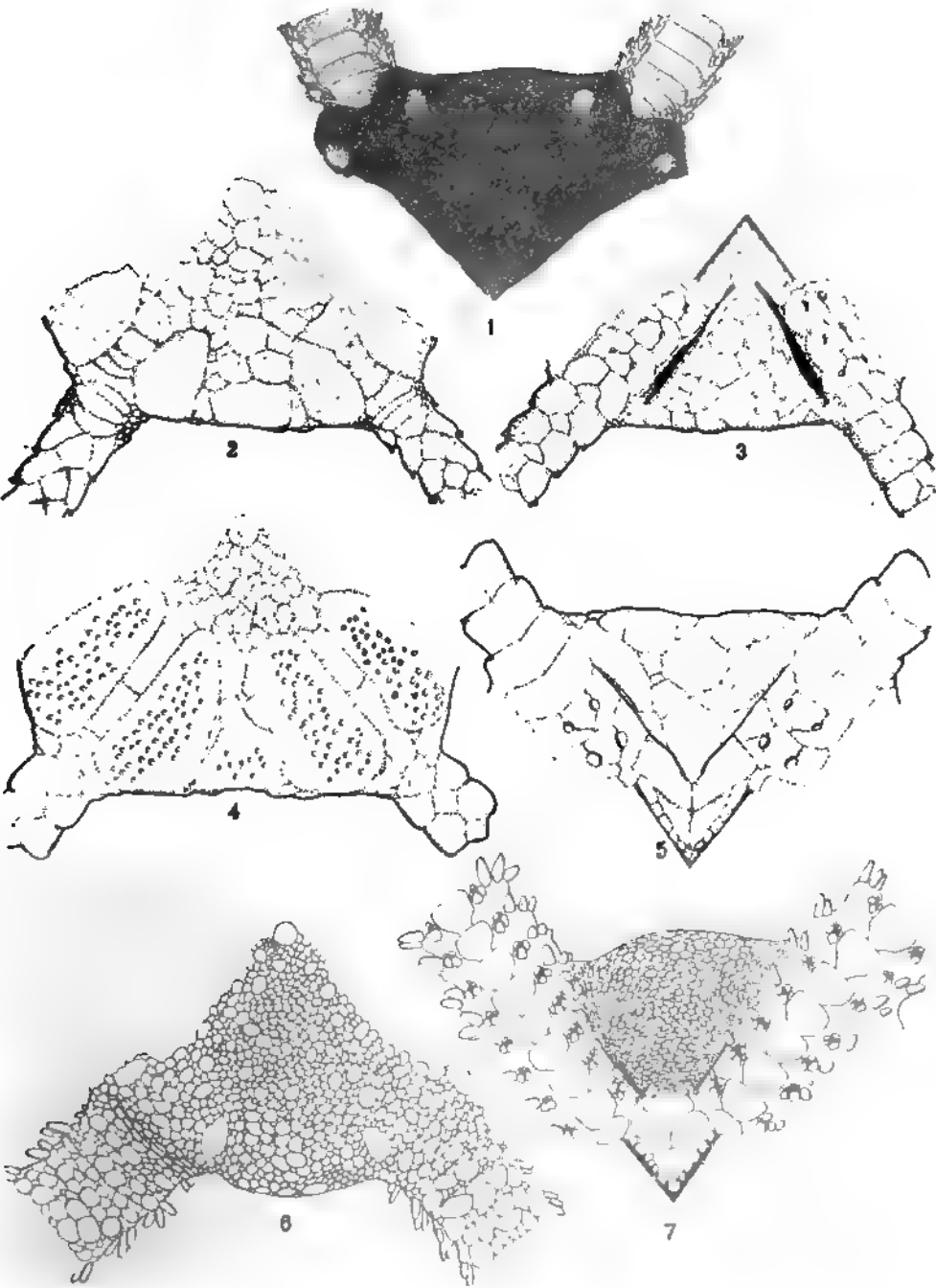
Verrill, A. E.

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EXPLANATION OF PLATES.

PLATE 1.

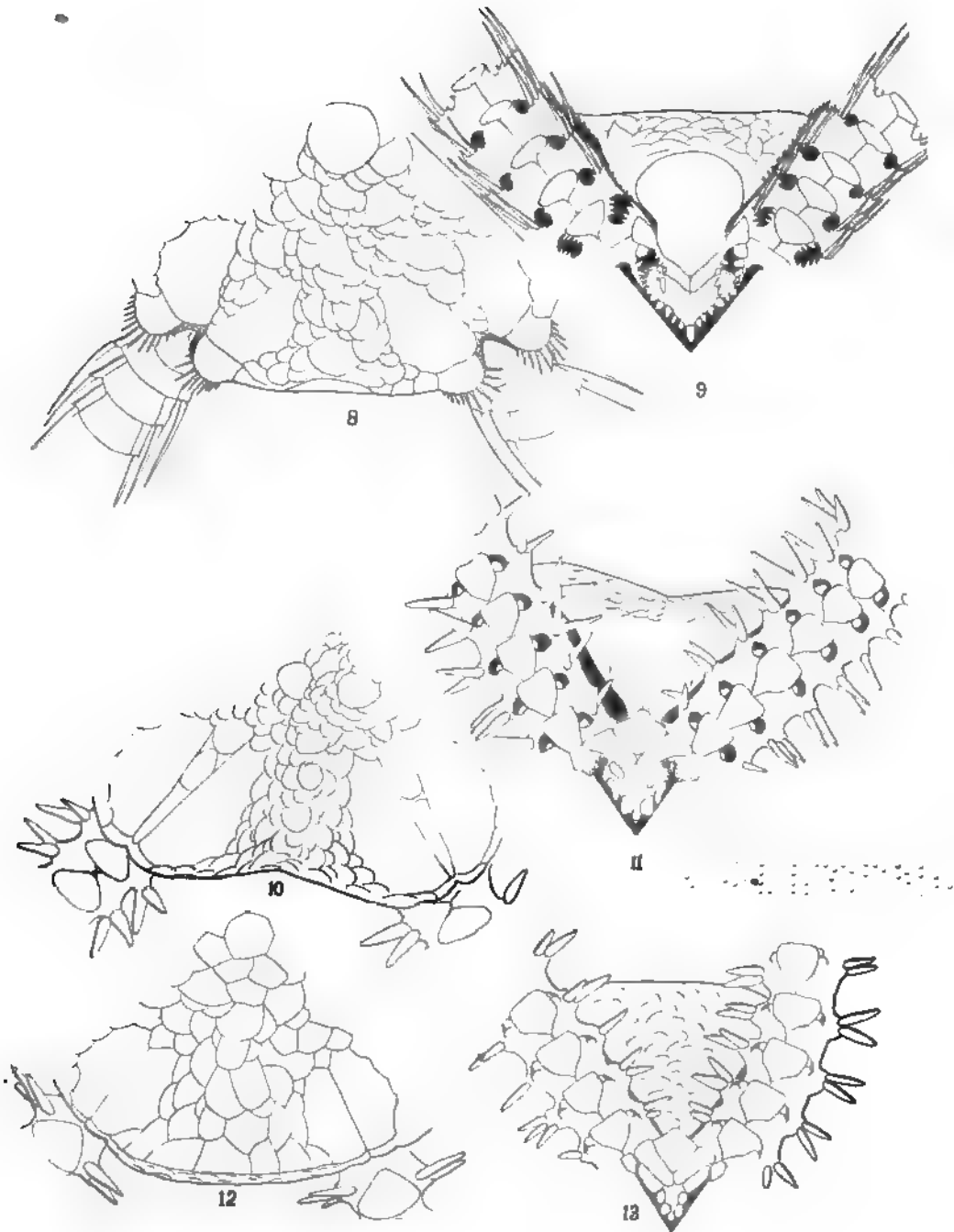
- Fig. 1. *Ophioderma panamensis* Ltk., partial dorsal view.
- Fig. 2. *Ophiomusium jolliensis* sp. nov., partial dorsal view.
- Fig. 3. Partial ventral view of same.
- Fig. 4. *Ophiomusium lymani* W. Thos., partial dorsal view.
- Fig. 5. Partial ventral view of same.
- Fig. 6. *Ophioplocus esmarki* Ly., partial dorsal view.
- Fig. 7. Partial ventral view of same.



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114 114

PLATE 2.

- Fig. 8. *Ophuira kofoidi* sp. nov., partial dorsal view.
Fig. 9. Partial ventral view of same.
Fig. 10. *Amphiura verrilli*, sp. nov., partial dorsal view.
Fig. 11. Partial ventral view of same.
Fig. 12. *Amphipholis pugetana* Ly., partial dorsal view.
Fig. 13. Partial view of same.



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PLATE 3.

Fig. 14. *Ophiecten pacificum* L. & M., partial dorsal view.

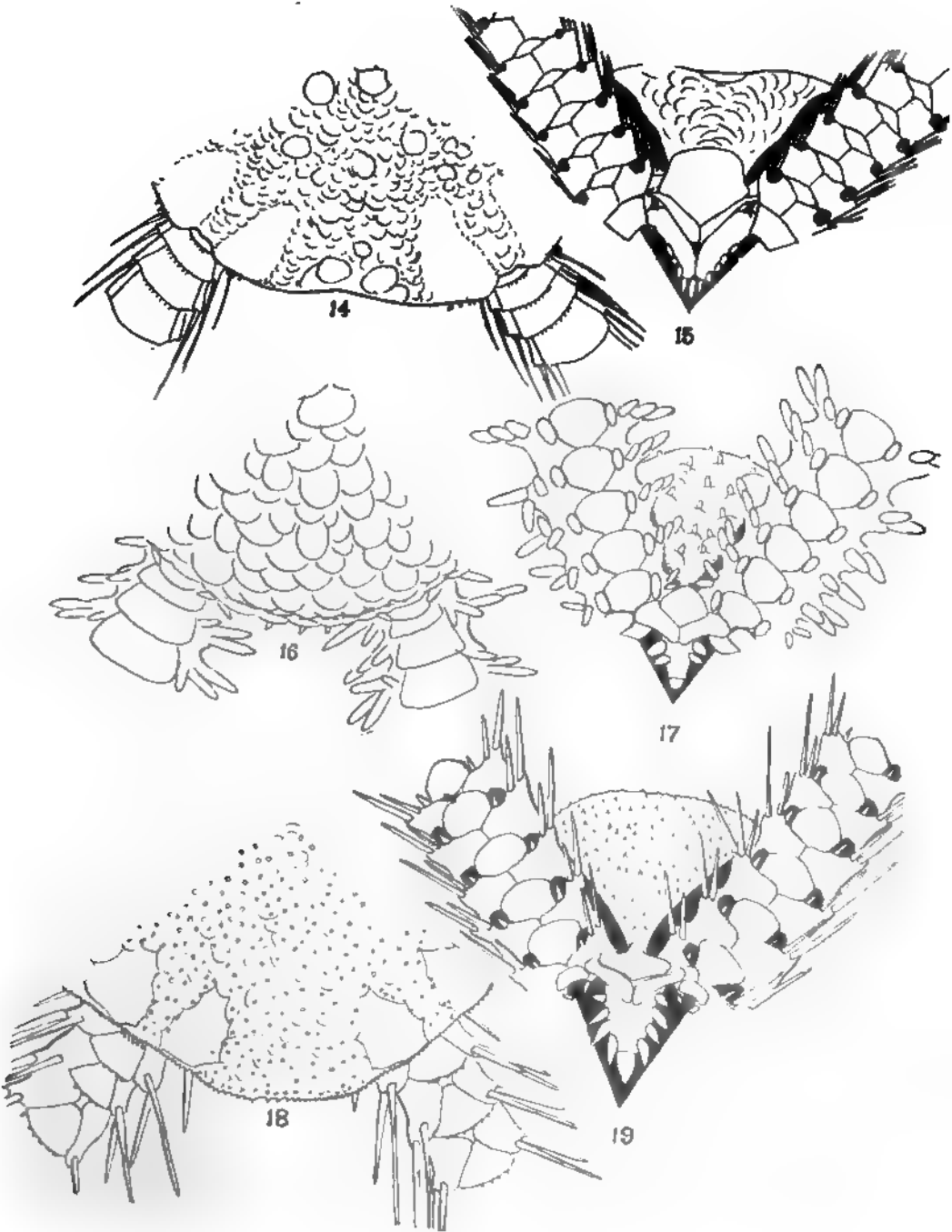
Fig. 15. Partial ventral view of same.

Fig. 16. *Ophiactis arenosa* Ltk., partial dorsal view.

Fig. 17. Partial ventral view of same.

Fig. 18. *Ophiacantha normani* Ly., partial dorsal view.

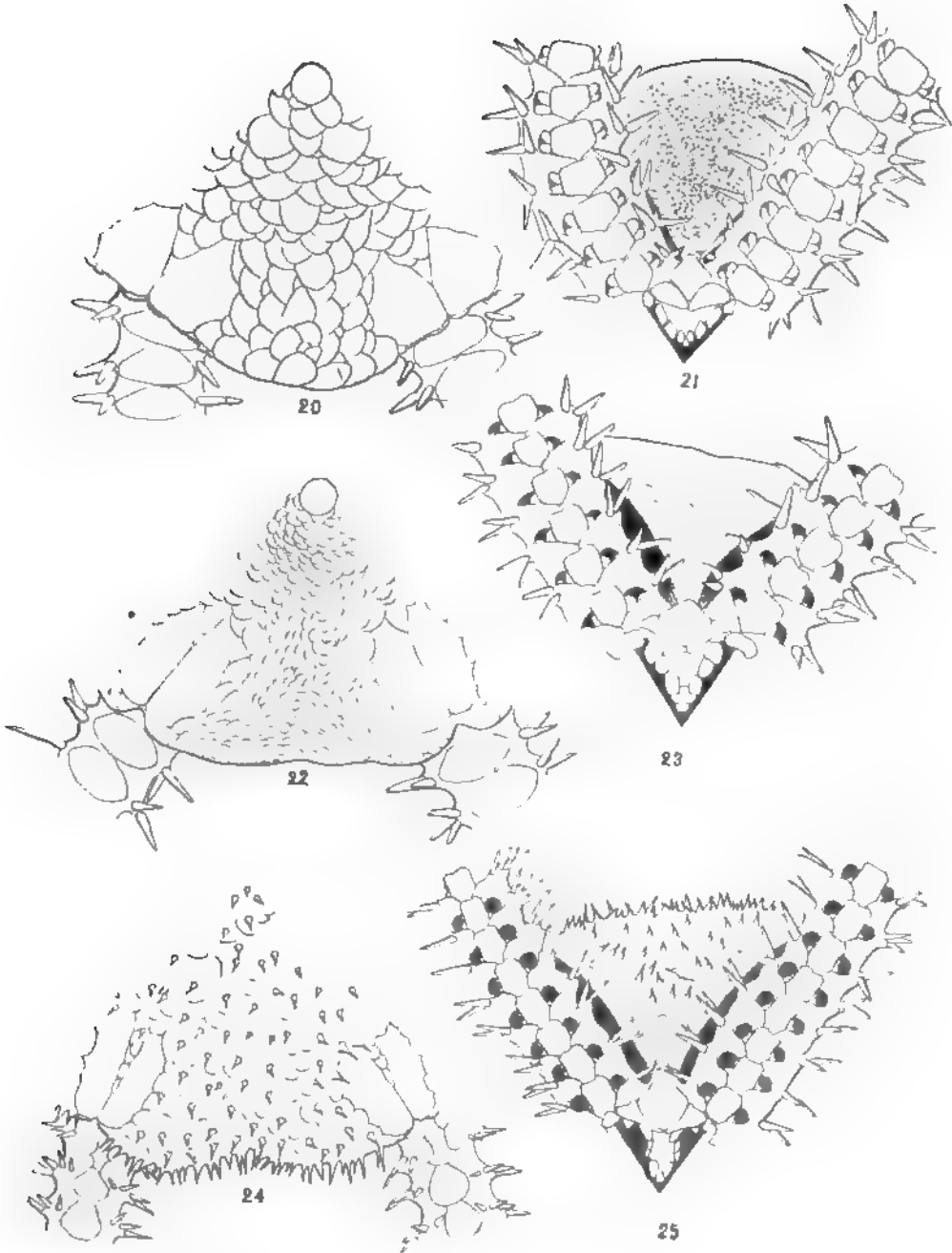
Fig. 19. Partial ventral view of same.



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PLATE 4.

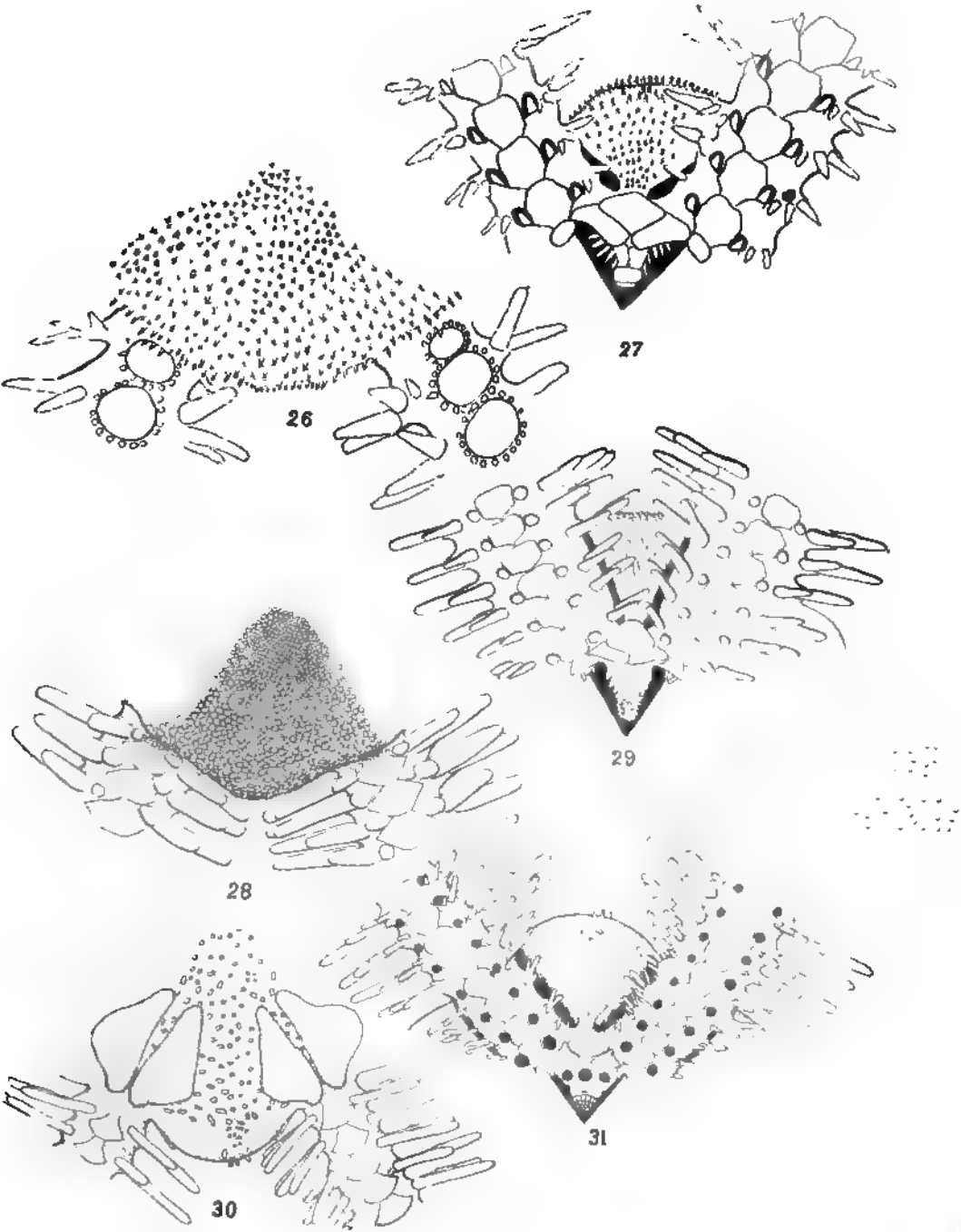
- Fig. 20. *Amphipholis puntarenae*, partial dorsal view.
Fig. 21. Partial ventral view of same.
Fig. 22. *Amphiodia barbarae* Ly., partial dorsal view.
Fig. 23. Partial ventral view of same.
Fig. 24. *Ophiocnida amphacantha*, sp. nov., partial dorsal view.
Fig. 25. Partial ventral view of same.



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PLATE 5.

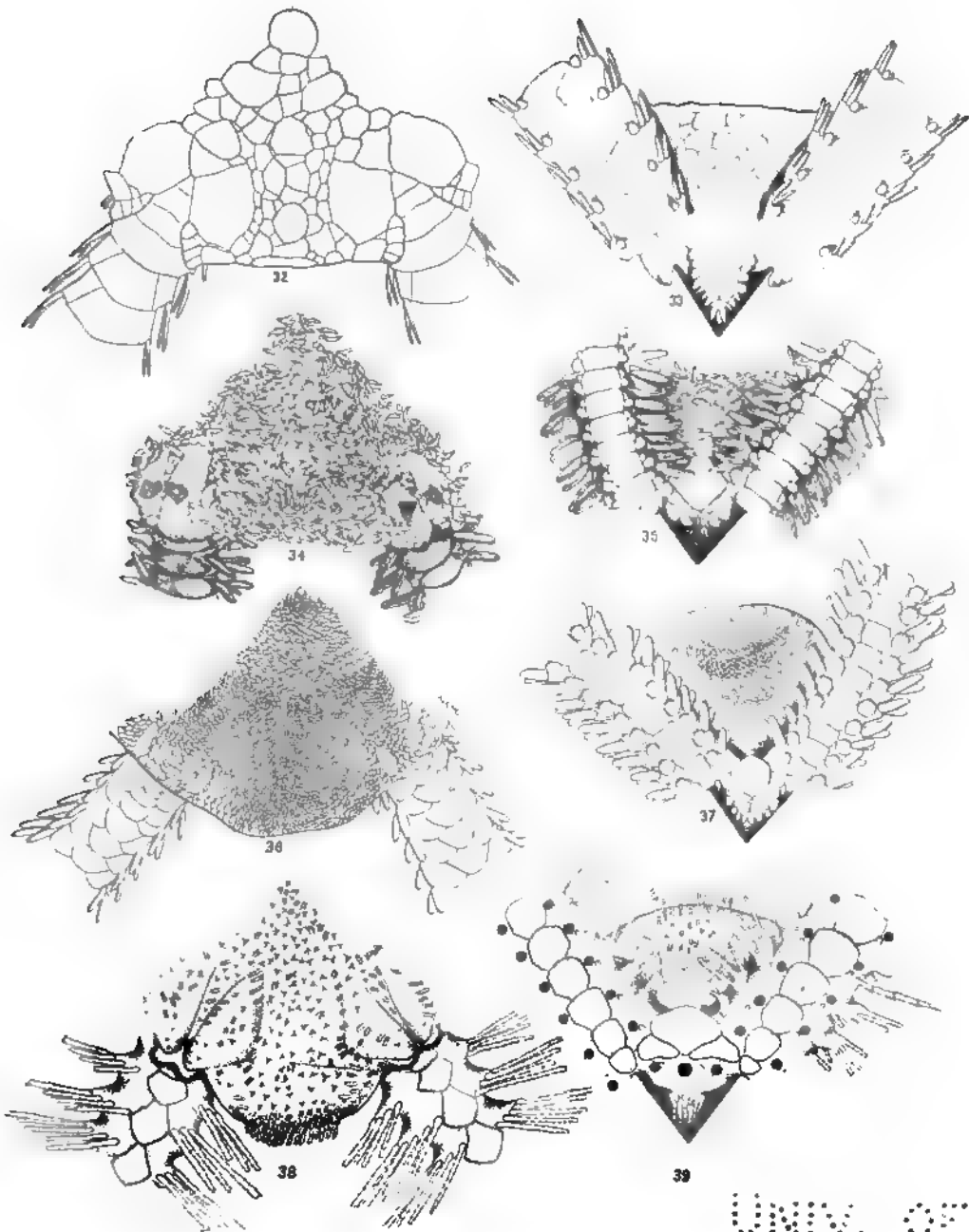
- Fig. 26. *Ophiopholis bakeri* sp. nov., partial dorsal view.
Fig. 27. Partial ventral view of same.
Fig. 28. *Ophiopteris papillosa* Ly., partial dorsal view.
Fig. 29. Partial ventral view of same.
Fig. 30. *Ophiothrix rudis* Ly., partial dorsal view.
Fig. 31. Partial ventral view of same.



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PLATE 6.

- Fig. 32. *Ophuira lütkeni* Ly., partial dorsal view.
Fig. 33. Partial ventral view of same.
Fig. 34. *Ophiocnida hispida* Le Conte, partial dorsal view.
Fig. 35. Partial ventral view of same. .
Fig. 36. *Ophionereis annulata* Le Conte, partial dorsal view.
Fig. 37. Partial ventral view of same.
Fig. 38. *Ophiothrix spiculata* Le Conte, partial dorsal view.
Fig. 39. Partial ventral view of same.



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[Reprinted from SCIENCE, N. S., Vol. XXXIII., No. 851, Pages 629-630, March 21, 1911.]

THE RELATION OF PERMEABILITY CHANGE TO CLEAVAGE, IN THE FROG'S EGG

UNFERTILIZED eggs (taken from the uterus) of the wood frog, *Rana sylvatica*, were caused to assume the normal orientation in the jelly, and to segment, by electrical stimulation. An alternating current of 60 cycles and 110 volts was passed through the tap water containing the eggs, from platinum electrodes about two inches apart. Stimulation for one second seemed to give the best results. The eggs were placed in fresh water immediately after stimulation.

Similar eggs were caused to segment by mechanical stimulation, even while the jelly remained intact. However, the most reliable mechanical means of inducing cleavage was found to be Bataillon's method of pricking the egg with an extremely fine needle. The first cleavage furrow often passed through the point of puncture.

Thousands of eggs were operated on. Control eggs were kept to both sets of experiments, and showed no segmentation or rotation within the jelly.

The following indirect evidence is given to show that a change in permeability is associated with both of these means of inducing cleavage:

1. These "stimuli," if applied in greater intensity or duration than is necessary to produce cleavage, result in rapid osmotic exchange with the medium and death of the egg.

2. Similar electrical and mechanical "stimuli" produce segmentation in the sea-urchin's

egg, a process which I have shown to be preceded by an increase in permeability.

With the exception of the rate of oxidation, this change in permeability is the only known common intermediate step between fertilization or artificial "stimulation," on the one hand, and cleavage on the other. Furthermore, there is indirect evidence to show that increase in permeability is associated with fertilization, in the frog's egg, as I have shown to be the case in the sea-urchin's egg: Backman and Runnström¹ observed that, whereas the osmotic pressure (freezing point lowering) of the ripe ovarian egg of the frog is the same as that of frog's serum, the osmotic pressure of the fertilized egg is the same as that of the pond water in which it lies. Since the frog's egg does not swell enormously after oviposition, it is improbable that the fall in osmotic pressure is due to the absorption of water. The simplest explanation is that the egg is, at this time, permeable to the internal osmotic substances. That this permeability is only a temporary condition is indicated by the fact that the osmotic pressure of the resulting embryo rises until it reaches that of frog's serum.

In conclusion, I wish to thank the Carnegie Institution, and especially Dr. Chas. B. Davenport, the director of the laboratory.

J. F. McCLENDON

STATION FOR EXPERIMENTAL EVOLUTION,
COLD SPRING HARBOR,
LONG ISLAND, N. Y.,
April 3, 1911

¹ *Biochem. Zeitschr.*, 1909, XXII., 390.

(From the Histological Laboratory of Cornell University Medical
College, New York City.)

Ein Versuch, amöboide Bewegung als Folgeerscheinung des wechselnden elektri- schen Polarisationszustandes der Plasma- haut zu erklären.

Von

J. F. McClendon.

(Mit 4 Textfiguren.)

Bonn, 1911.

Separat-Abdruck aus dem Archiv für die ges. Physiologie Bd. 140.

Verlag von Martin Hager.

(From the Histological Laboratory of Cornell University Medical College,
New York City.)

Ein Versuch, amöboide Bewegung als Folgeerscheinung des wechselnden elektrischen Polarisations- zustandes der Plasmahaut zu erklären.

Von

J. F. McClendon.

(Mit 4 Textfiguren.)

Nach der Ansicht vieler Autoren werden amöboide Bewegungen durch Änderungen der Spannung der Oberfläche hervorgerufen. Dieser Spannungszustand der Oberfläche kann entweder eine echte Oberflächenspannung oder, nach R h u m b l e r¹⁾ zuweilen eine Spannung des Ektoplasmas als Folge von Wasserverlust („Gelatinierungsspannung oder Gelatinierungsdruck“) sein. In dieser Mitteilung soll nur von echter Oberflächenspannung die Rede sein.

Lässt man Kaliumbichromat gegen einen Tropfen Quecksilber in verdünnter Salpetersäure diffundieren, dann sendet der Quecksilbertropfen von der Stelle, die zuerst mit dem K_2CrO_4 in Berührung tritt, einen amöboiden Fortsatz aus. Es findet nämlich unter Verminderung der Oberflächenspannung eine Bewegung der äusseren Schichten von dem Bichromat weg und eine solche der inneren gegen das letztere zu statt. Ähnliche Strömungen wurden bei der Bewegung vieler Amöben beobachtet. Hier verursachen die Rückströmungen an der Oberfläche und die Vorwärtsströmungen im Inneren naturgemäss einen allmählichen Austausch von Ekto- und Endoplasma. Dieser Vorgang, den R h u m b l e r den „Ektoendoplasma-

1) L. R h u m b l e r, Zur Theorie der Oberflächenkräfte der Amöben. Zeitschr. f. wissensch. Zool. Bd. 83, S. 1. 1905.

prozess nennt, ist schon vor Jahren von Wallich und Montgomery¹⁾ (1879, 1881) beobachtet worden.

Nach Jennings²⁾ findet ein derartiges Abfließen der vorgeschobenen Oberfläche nicht bei allen Amöben statt. Doch liesse sich dessen Ausbleiben in solchen Fällen durch die Annahme einer zäheren Plasmahaut und Alveolarwandsubstanz des Protoplasmas genügend erklären. Das oberflächliche Abfließen wäre dann teilweise auf die einzelnen Alveolen beschränkt. Rhumbler jedoch erklärt dieses Ausbleiben der oberflächlichen Strömungen auf Grund von Berthold's Theorie dahin, dass die Fortbewegung der Amöbe durch einseitiges Anhaften an der Unterlage hervorgerufen wird.

Zweifellos hat die Plasmahaut einen Einfluss auf den Mechanismus der Bewegung. Nach Quincke ist dieselbe ein Fett-, nach Overton ein lipoides Häutchen, doch ist ihre wirkliche Zusammensetzung noch nicht sichergestellt. Sie ist in Wasser unlöslich, jedoch für Wasser und andere, namentlich lipoidlösliche Substanzen durchlässig. Das Cytoplasma lebender Zellen oder Eier enthält Lipoideiweissverbindungen, die in Wasser nicht löslich sind, sich aber bei Berührung mit Wasser unter Freiwerden von Lipoiden zu zersetzen scheinen [McClendon³⁾, 1910]. Nach dem Gibbs'schen Prinzip sammeln sich diese Lipoiden an der Oberfläche und bilden eine Schicht, die eine weitere Zersetzung der Lipoideiweissverbindungen verhindert. Dieses lipoiden Häutchen ist unter gewöhnlichen Umständen von ultramikroskopischer Dicke; wird hingegen dem Wasser etwas Alkohol zugefügt, so kann unter Umständen ein verhältnismässig dickes Häutchen auf der Oberfläche (z. B. von Hühnereidotter) geformt werden.

Dass die Plasmahaut der Amöbe für Anionen weniger durchlässig zu sein scheint als für Kationen, ist eine Vermutung, die durch zwei Tatsachen nahegelegt wird:

1) E. Montgomery, Elementary Functions and Primitive Organization of Protoplasm. St. Thos. Hos. Repts., London 1878, n. s. IX. 1878. — Zur Lehre von der Muskelkontraktion. II. Die amöboide Bewegung. Pflüger's Arch. Bd. 25 S. 499.

2) H. S. Jennings, Contributions to the Study of the Behavior of Lower Organisms. VI. Publ. Carnegie Instit. Washington Nr. 16 p. 129. 1904.

3) J. F. McClendon, Dynamics of Cell Division. II. Americ. Journ. of Physiol. vol. 27 p. 240. 1910.

1. Wird durch einen Wassertropfen, in dem eine Amöbe schwimmt, ein schwacher elektrischer Strom geleitet, so wandert das Tier passiv gegen die Anode.

2. Wird ein starker elektrischer Strom durch das Wasser geleitet, dann beginnt der Zerfall der Amöbe am anodalen Pol.

Der zerstörende Einfluss eines elektrischen Stromes auf lebendes Gewebe ist vielleicht die Folge einer Anhäufung von Ionen, die in ihrer freien Bewegung durch gewisse Gewebsstrukturen behindert werden. Da nun an der Amöbe die Zerstörungserscheinungen zuerst an der Oberfläche auftreten, so dürfte die Plasmahaut für eine Behinderung der Ionenwanderung verantwortlich zu machen sein; die Ionen würden sich, da sie die Plasmahaut nicht leicht passieren können, hinter derselben anhäufen, und diese Anhäufung würde die sichtbaren Erscheinungen des Zerfalles hervorrufen.

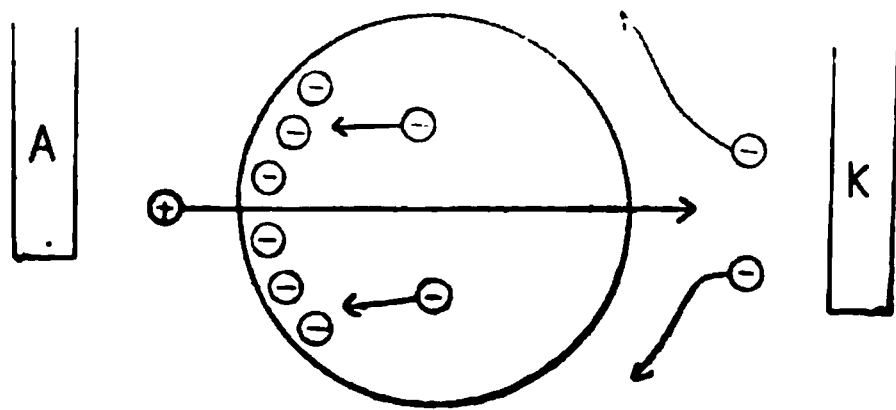


Fig. 1. Schematische Darstellung des Einflusses eines elektrischen Stromes auf eine Amöbe. A = Anode. K = Kathode. Der grosse Kreis stellt die Plasmahaut dar, die kleinen Kreise Ionen mit ihrer entsprechenden Ladung. Die Pfeile deuten die Bewegungsrichtung der Ionen an.

Der Zerfall der oberflächlichen Schichten tritt nicht an beiden Polen der Amöbe gleichzeitig auf, sondern zunächst nur an der Anode, so dass es den Anschein hat, als ob die Plasmahaut der Durchwanderung der Anionen einen grösseren Widerstand entgegengesetzt als der der Kationen. Die Fig. 1 stellt diese Annahme schematisch dar. Der grosse Kreis repräsentiert die Plasmahaut, die kleineren die Ionen, deren Ladung durch + oder — bezeichnet ist. Die Bewegungsrichtung der Ionen ist durch Pfeile angedeutet. Die positiven Ionen scheinen die Amöbe ungehindert zu passieren, während die negativen ausserhalb des Zelleibes denselben leicht umgehen können. Jene negativen Ionen jedoch, die im Protoplasma eingesperrt sind, scheinen die Oberhaut nicht passieren zu können, stauen sich daher hinter derselben und rufen dann die gewissen Auflösungserscheinungen hervor.

Wird jedoch ein Strom, der zu schwach ist, um das Plasma zu zerstören, durch das Wasser geleitet, dann wandert die Amöbe passiv gegen die Anode, eine Beobachtung, die Hirschfeld's¹⁾ (1909) Annahme einer positiven Ladung derselben nicht bestätigt. Diese passive Wanderung der Amöbe könnte in folgender Weise erklärt werden. Im Innern derselben wird ununterbrochen ein Elektrolyt erzeugt, dessen Anionen die Plasmahaut nicht passieren können. Diese geben der Amöbe eine negative Ladung, so dass das Tier, wenn ein Strom durch das Wasser geleitet wird, gegen die Anode gezogen wird.

Da unter allen Elektrolyten, die unter solchen Umständen erzeugt werden könnten, die Kohlensäure wohl der ausgiebigste ist, dürfte deren Gegenwart den oben gestellten Forderungen genügen.

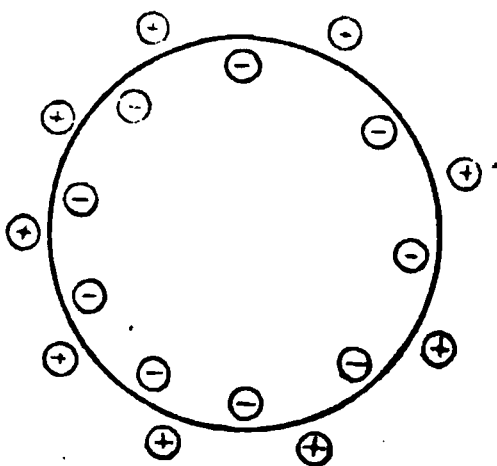


Fig. 2. Schematische Darstellung des elektrischen Polarisationszustandes der Amöbenoberfläche als Resultat einer relativen Undurchlässigkeit der Plasmahaut für die Anionen der Kohlensäure. Zeichen wie in Fig. 1.

Es ist allerdings wahr, dass das elektrolytische Dissoziationsvermögen der Kohlensäure sehr gering ist. Dies würde dann eben eine starke Konzentration derselben nötig machen, die möglicherweise in der Zelle existiert. Wir können nun annehmen, dass die H^+ -Ionen aus der Amöbe herauswandern können, während die HCO_3^- - und CO_3^{--} -Ionen innerhalb derselben zurückgehalten werden und ihr die nötige negative Ladung geben. Diese Scheidung der Ionen würde noch durch die grössere Schnelligkeit, mit der die H^+ -Ionen durch das Plasma wandern, gefördert. Die Anionen im Inneren und jene Kationen, die durch sie auf der Oberfläche zurückgehalten werden (Fig. 2), bewirken eine Polarisation der letzteren, ähnlich dem Lippmannschen Phänomen im Kapillarelektrometer. Infolge dieser Polarisation muss die Oberflächenspannung eine geringe sein, wie es tatsächlich

1) L. Hirschfeld, Ein Versuch, einige Lebenserscheinungen der Amöben physikalisch-chemisch zu erklären. Zeitschr. f. allgem. Physiol. Bd. 9 S. 529. 1909.

die Beobachtung lehrt. Obzwar die Oberfläche einer Amöbe möglicherweise aus Lipoiden besteht, besitzt das Tier eine viel geringere Oberflächenspannung als ein Öltropfen im Wasser.

Unter solchen Umständen würden amöboide Bewegungen durch örtliche Veränderungen der Oberflächenspannung erzeugt, die ihrerseits eine Folge der wechselnden Oberflächenpolarisation sind. Während die CO_3^{--} -Ionen vielleicht nicht leicht passieren können und die Kationen unter ihrem Einflusse zum Teil zurückgehalten werden, könnten andererseits die nicht dissoziierten Moleküle als CO_2 auswandern, da CO_2 in Lipoiden löslich ist. Der Polarisationszustand könnte dann nur so lange aufrechterhalten werden, als CO_2 innerhalb der Amöbe in gleichem Maasse erzeugt wird, als sie auswandert. Würde an irgendeiner Stelle die Kohlensäurebildung steigen oder fallen, dann würde die gleichzeitige Polarisationsveränderung des nächstliegenden Teiles der Oberfläche spontane Bewegung erzeugen. Die Kohlensäurebildung wird vielleicht zeitweise durch äussere Bedingungen beeinflusst, z. B. durch Substanzen, die von anderen Organismen in das Wasser abgegeben werden. Eine Erhöhung der CO_2 -Bildung würde dann positiven Tropismus hervorbringen.

Andererseits würde eine Erhöhung der Durchlässigkeit der Plasmahaut eine Herabsetzung der Polarisation derselben zur Folge haben. In einer früheren Arbeit (1910) habe ich eine Reihe von Agentien aufgezählt, die die Durchlässigkeit der Plasmahaut für Anionen erhöhen. Dieselben Agentien bewirken auch in der Amöbe eine Erhöhung der Oberflächenspannung, die sich in einer grösseren Abrundung des Körpers äussert. Wird die Amöbe nur auf einer Seite von diesen Agentien beeinflusst, dann zeigt sie negativen Tropismus. Negativer Tropismus kann dadurch erklärt werden, dass die ihn bewirkenden mechanischen, thermischen, chemischen u. dgl. Einflüsse die Plasmahaut an der der Einwirkung nächstgelegenen Stelle auflockern; der daraus resultierende Verlust an Polarisation unter gleichzeitiger Erhöhung der Oberflächenspannung veranlasst die Amöbe, sich von dem schädlichen Einfluss zurückzuziehen.

Der Teil der Oberfläche, an dem die Plasmahaut verändert worden ist, bleibt aber nicht depolarisiert, da er seine frühere Undurchlässigkeit zurückerhält, sobald sich die Amöbe dem schädigenden Einfluss entzogen hat.

Die Polarisation ist nicht der einzige Faktor, der die Ober-

flächenspannung zu verändern vermag. Es ist z. B. eine bekannte Tatsache, dass Seifen die Oberflächenspannung an Lipoiden vermindern. Wenn nun die Plasmahaut aus Lipoiden besteht, sollte man [mit Bütschli, J. Loeb, Robertson, Michaelis u. a.] vermuten, dass Seifen eine Herabminderung der Oberflächenspannung an der lebenden Zelle bewirken; doch habe ich das gerade Gegenteil beobachtet. Eine Kapillarröhre wurde mit fester Seife oder Seifenlösungen von verschiedener Konzentration gefüllt und mittels der „mechanischen Hand“ [McClendon¹⁾, 1909] so weit in Wasser eingetaucht, bis ihre feine Öffnung ganz in die Nähe einer grossen *Amoeba proteus* kam. Das Tier zeigte immer deutlichen negativen Chemotropismus, in dem es rasch von der Seife, die aus der Kapillarröhre heraussickerte, wegwanderte. Manchmal war die Bewegung nicht rasch genug, um das Leben des Tieres zu retten. Leider konnte der genaue Zeitpunkt, an dem die ersten Seifenmoleküle die Amöbe erreichten, nicht bestimmt werden. So liess sich daher auch nicht feststellen, ob ein kurzdauernder Vorstoss des Tieres gegen die Seifenlösung zu, wie er manchmal beobachtet wurde, als eine „Reiz-“ oder spontane Bewegung aufzufassen sei. Auf jeden Fall können wir schliessen, dass der hauptsächlichste Einfluss, den die Seife auf die Amöbe ausübt, geeignet ist, die Durchlässigkeit der Oberfläche für Anionen zu erhöhen, da das Tier negativen Tropismus zeigt.

In einer kürzlich erschienenen interessanten Arbeit über die Rolle der verschiedenen Kolloidaltzustände der Oberfläche der Amöben bei Nahrungsaufnahme kam Rhumbler²⁾ (1910) zu dem Schlusse, dass die Annahme einer festen, elastischen und relativ dicken Oberflächenschicht notwendig ist, um jene Form der Nahrungsaufnahme zu erklären, dass er „Circumvallation“ nennt. Er vermutet, dass „Circumvallation“ durch eine örtliche Auflösung der halbfesten Ober-

1) J. F. McClendon, Protozoan Studies I. Reactions of *Amoeba Proteus* to Minutely Localized Stimuli. Journ. of exper. Zool. vol. 6 p. 265. — Autoreferat über vorstehende Abhandlung in Arch. f. Entwicklungsmechanik Bd. 37 S. 323. 1909. — The Reaction of *Amoeba* to Stimuli of Small Area. Americ. Journ. Physiol. vol. 21. 1908. Proc. Americ. Physiol. Soc. 1907 p. 13.

2) L. Rhumbler, Die verschiedenartigen Nahrungsaufnahmen bei Amöben als Folge verschiedener Kolloidaltzustände ihrer Oberflächen. Arch. f. Entwicklungsmechanik Bd. 30 S. 194. 1910.

flächenschicht durch einen von der Beute ausgeübten „Reiz“ eingeleitet wird ¹⁾).

Es gibt jedoch eine Art der Nahrungsaufnahme, die der „Circumvallation“ zwar verwandt, aber in folgender Weise durch Veränderungen der Oberflächenspannung erklärlich ist: wenn z. B. eine

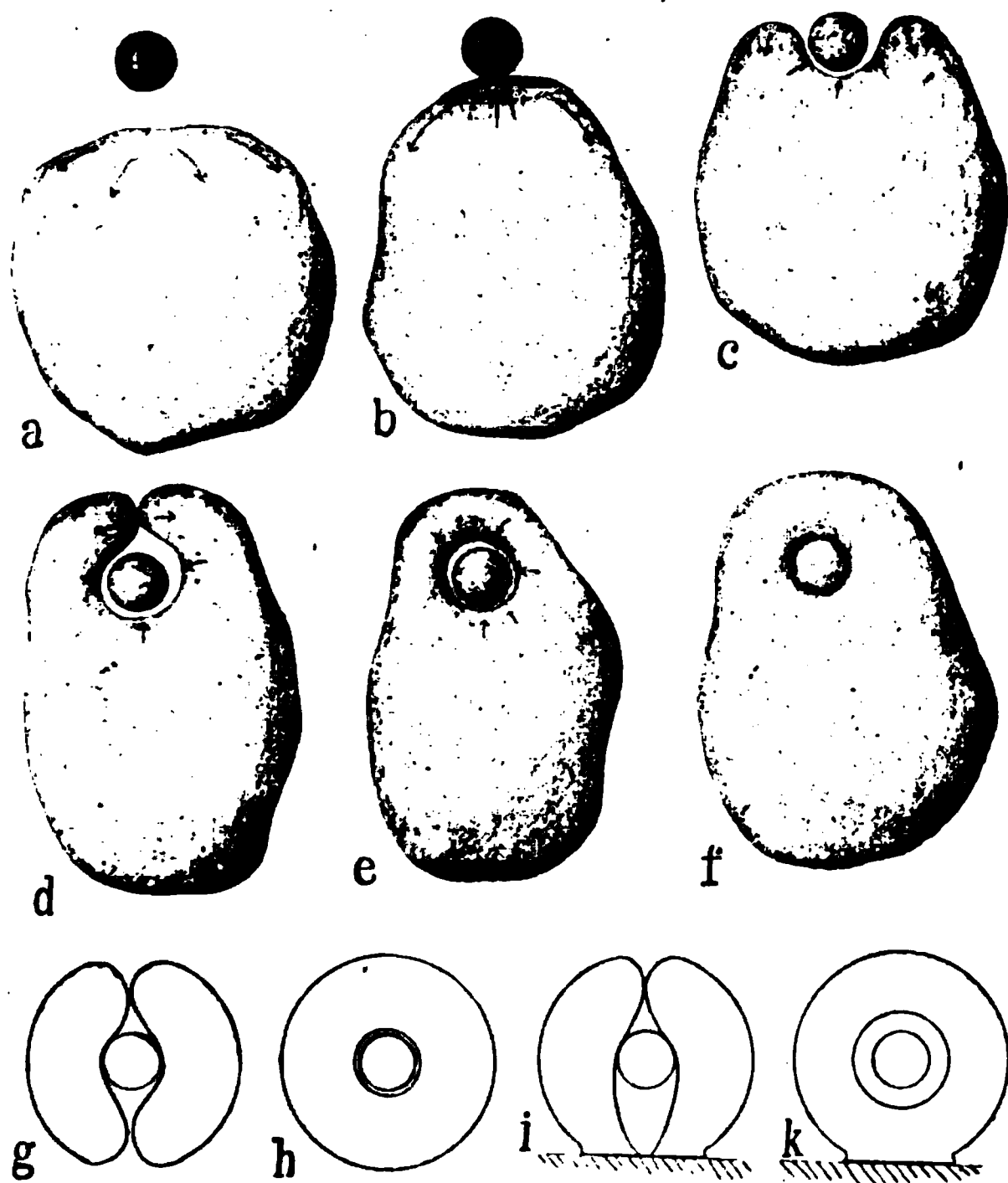


Fig. 3. Schematische Darstellung der Nahrungsaufnahme einer Amöbe. Die Pfeile deuten die Richtung an, in der eine Erhöhung der Oberflächenspannung erfolgt. Der kleine Kreis stellt eine Algenzelle dar. *g-k* = Querschnitte durch den Plasmaring der Figuren *e-f*.

Amoeba proteus sich in der Nähe einer Algenzelle befindet (Fig. 3a), dann bewirken auf Grund meiner früheren Voraussetzungen

1) Es ist zweifellos, dass eine feste Oberfläche die Nahrungsaufnahme der Amöbe nicht verhindert. Schaudinn (1899) (Generationswechsel von *Trichosphaerium Sieboldi*. Anhang d. Abhandl. Berliner Akad. d. Wissensch. 1899

1) beobachtete die Aufnahme von fester Nahrung bei *Trichosphaerium Sieboldi*, dessen Körper von einer Gallerthülle umgeben ist, in die zahlreiche liär gestellte Stäbchen, $MgCO_3$, eingebettet sind. Eine zum mindest halbstarre flächenschicht ist, wie Rhumbler beweist, sogar notwendig, um die Nahrungsaufnahme durch „Invagination“ zu ermöglichen.

Stoffe, die aus der Zelle ausgeschieden werden, eine Steigerung der CO_2 -Erzeugung im nächstliegenden Teil des Amöbenleibes; denn die Amöbe breitet sich gegen die Alge aus. Manchmal rollt sie die letztere sogar etwas vor sich her (Fig. 3 *b*). Die Berührung mit der Alge oder die stärkere Konzentration der reizenden Substanzen verursacht eine lokale Erhöhung der Durchlässigkeit und der Spannung eines Teiles der Plasmahaut, welcher das Feld verminderter Spannung durchschneidet (Fig. 3 *c*). Die seitlichen Partien schieben sich nun weiter nach vorne, bis sie die Beute ganz umgeben (Fig. 3 *d*) und sich dann vor derselben vereinigen (Fig. 3 *e*). Die Beute ist dann von einem Protoplasmaring eingeschlossen. Die Oberflächenspannung bestrebt nun eine Verkleinerung des letzteren. Manchmal kann der dabei von allen Seiten einwirkende Druck ein Herauspressen der Beute nach oben zur Folge haben, so dass die Nahrungsaufnahme misslingt. Unter günstigen Bedingungen hingegen schliesst sich der Ring über und unter dem Fremdkörper, wie es im Querschnitt in Fig. 3 *g* und *h* dargestellt ist; gleichzeitig wird auch etwas Wasser mit der Beute eingeschlossen, und zwar dürfte, wenn die Amöbe an der Unterlage anhaftet, etwas mehr Wasser eingeschlossen werden.

Diese Methode der Nahrungsaufnahme nimmt eine Zwischenstellung zwischen R h u m b l e r's Klassen „Circumfluenz“ und „Circumvallation“ ein, indem die Amöbe in Berührung mit der Beute kommt, zugleich aber Wasser mit der Nahrung aufnimmt.

Versuche, die ich an Seeigeleiern unternommen habe, unterstützen meine Ausführungen. Dass Eier amöboide Bewegungen ausführen können, ist eine bekannte Tatsache. P r o w a z e k¹⁾ (1903) beobachtete an Seeigeleiern, die in die Körperflüssigkeit einer Annelide gelegt wurden, aktive Bewegungen; ein Ei nahm eine „Eläocyte“ in sich auf.

Wenn ein allmählich stärker werdender elektrischer Strom durch eine isotonische Zuckerlösung geleitet wird, die Seeigeleier enthält, so muss man eine Erhöhung der Polarisierung in dem der Anode nächstgelegenen Teile der Plasmahaut erwarten, da die Anionen, die hinter der Membran zurückgehalten werden, eine Anhäufung der Kationen an der Aussenseite hervorrufen (Fig. 4 *a*). Die Anionen

1) P r o w a z e k, Studien zur Biologie der Zelle. Zeitschr. f. allg. Physiol. Bd. 2 S. 385. 1903.

des Wassers können das Ei umgehen, während die Kationen es durchwandern. Die Erhöhung der Polarisation muss eine Herabsetzung der Oberflächenspannung und damit eine Ausdehnung der Eioberfläche gegen die Anode zu veranlassen. Zahlreiche Beobachtungen bestätigten diese Vermutung, indem das Ei unter den gestellten Bedingungen jedesmal ein oder mehrere Pseudopodien gegen die Anode ausstreckte. In einigen Fällen wurde auch ein Abfließen der oberflächlichen Schichten bemerkt.

Das beschriebene Phänomen war von sehr kurzer Dauer, da das vorgestreckte Cytoplasma bald zu desintegrieren begann und osmo-

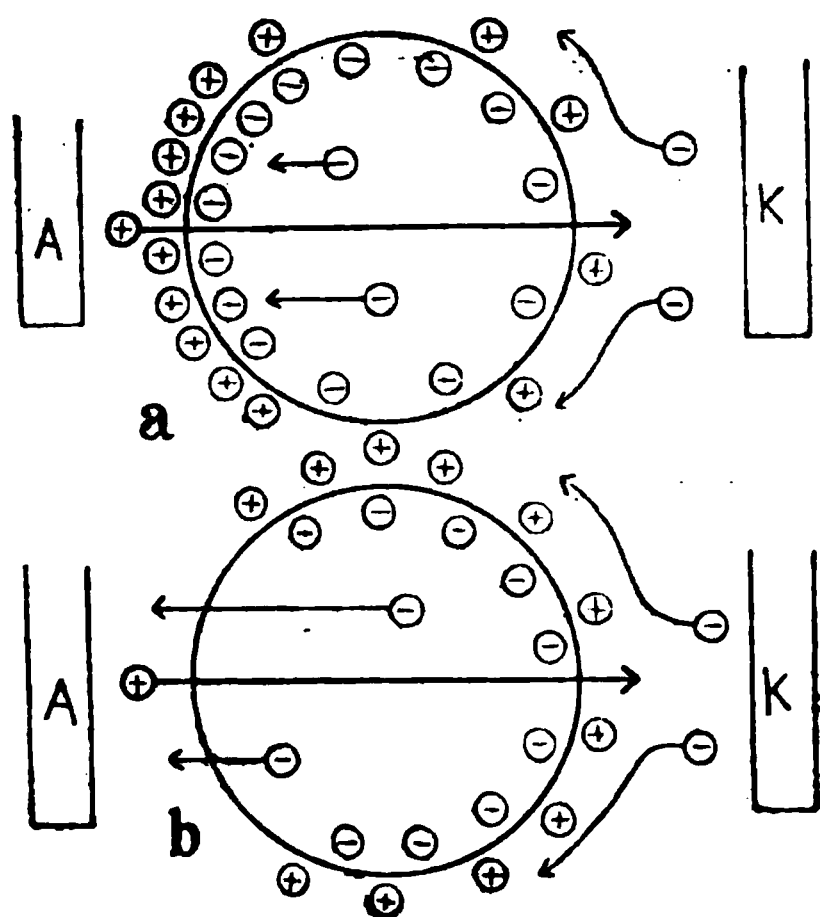


Fig. 4. Schematischer Versuch, den Tropismus der Amöbe gegen die Kathode zu erklären. Zeichen wie in Fig. 1. (In der Amöbe äussert sich Galvanotropismus an der Kathode, Kataphorese an der Anode.)

tische Erscheinungen die Bewegungen auf Grund der Spannungsdifferenzen verhielten. Der Zersetzungsprozess wurde wahrscheinlich durch die Anhäufung der Anionen eingeleitet.

Während in einem schwachen Strom das Seeigeelei Pseudopodien gegen die Anode ausstreckt, zeigt die Amöbe Tropismus nach der Kathode. Ein starker Strom aber verursacht in beiden Formen eine Zersetzung am anodalen Pol. Diese Tatsache scheint anzudeuten, dass die Verminderung der Oberflächenspannung und der Zerfall des Plasmas im Seeigeelei durch ähnliche, in der Amöbe aber durch entgegengesetzte Faktoren hervorgerufen werden. Doch kann dieser anscheinende Widerspruch dadurch erklärt werden, dass auch in der Amöbe die Polarisation des anodalen Poles steigt; bevor jedoch ge-

nügend Zeit verstreicht, um eine Bewegung auszuführen, wird die Plasmahaut zerstört, so dass der Polarisationsgrad dieser Stelle unter das Normale der übrigen Oberfläche sinkt (Fig. 4 b). Der Schlusseffekt des Stromes wäre somit verminderte Polarisation der anodalen Gegend, und die Amöbe wandert gegen die Kathode.

Die erhöhte Durchlässigkeit eines Teiles der Plasmahaut würde die Polarisation der gesamten Oberfläche etwas herabsetzen, da die Anionen jenen weniger dichten Teil durchwandern könnten. Doch wird in der Amöbe ununterbrochen CO_2 erzeugt und wahrscheinlich um so energischer, je schneller sie weggeschafft wird, wie es ja bei allen Endprodukten chemischer Vorgänge der Fall ist. Aus diesem Grunde dürfte die Polarisation des nicht desintegrierenden Teiles der Oberfläche sehr wenig, wenn überhaupt, herabgesetzt werden.

Der Potentialabfall zwischen den beiden Seiten jenes Teiles der Plasmahaut, der der Kathode zunächst liegt, ist entgegengesetzt dem Potentialabfall, der an jener Stelle von den Elektroden erzeugt würde (Fig. 4 b). Man muss daher annehmen, dass die normale Polarisation der Plasmahaut einem Potentialabfall entspricht, der stärker ist als der des umgebenden Wassers, da sonst der erstere durch den letzteren unterdrückt würde.

Reprinted from the American Journal of Physiology.

VOL. XXIX. — JANUARY 1, 1912. — NO. III.

**THE INCREASED PERMEABILITY OF STRI-
ATED MUSCLE TO IONS DURING
CONTRACTION.**

By J. F. McCLENDON.

[FROM THE EMBRYOLOGICAL LABORATORY OF CORNELL UNIVERSITY MEDICAL
COLLEGE, NEW YORK CITY.]

THE INCREASED PERMEABILITY OF STRIATED MUSCLE TO IONS DURING CONTRACTION.

By J. F. McCLENDON.

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New York City.]*

ACCORDING to the membrane theory of Bernstein,¹ the muscle fibre is surrounded by a plasma membrane or surface film, allowing easier exit to one or more classes of kations than to the corresponding anions. The kations of some electrolyte which is more concentrated within than without come through the surface film and give the surface a positive electric charge. Destruction or alteration of this film or membrane causes a negative variation (the affective surface being less positive than the normal surface).

It has been suggested that potassium ions, which are more concentrated within the muscle than in the blood plasma, give the muscle the positive charge. However, one should then expect a reversal of the electric effects by placing muscle in an isotonic solution of potassium salts. This was shown by Höber² and Overton³ not to occur. R. Lillie⁴ suggests that lactic and carbonic acids are the electrolytes in question, and the H ions give the muscle surface the positive charge. The difficulty with this view lies in the fact that muscle, as shown by Overton, is in general permeable to substances soluble in many oils. It seems, therefore, that this question is not settled.

If the negative variation or "action current" of muscle is due to increased permeability to any ions, we should expect increased electric conductivity on contraction. It has long been known that the electric

¹ BERNSTEIN: *Archiv für die gesammte Physiologie*, 1902, xcii, p. 521.

² HÖBER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 607.

³ OVERTON: *Sitzungsberichte der physikalisch-medizinische Gesellschaft, Würzburg*, 1905, p. 2.

⁴ LILLIE: *This journal*, 1909, xxiv, p. 14; 1911, xxviii, p. 197.

conductivity of muscle increases at death. Du Bois Reymond⁵ explained the electric resistance of living muscle by the presence of membranes, which become altered (permeable) at death. He showed that muscle becomes polarized on the passage of an electric current. It seems to me that Kodis⁶ and Galaeotti⁷ take a step backward in attributing the increased conductivity of dead muscle to the liberation of ions. Galaeotti tried to support his view by determinations of the freezing points of living and dead muscle, but found, on the contrary, that the change in electric conductivity did not correspond to the change in the osmotic pressure.

I have not found in the literature a clear statement that the electric conductivity of muscle has ever been measured, actually, during contraction. The contraction period (about one fifth of a second for frog's muscle) is too short for an accurate measurement of conductivity to be made. Therefore I decided to measure the conductivity during tetanus.

EXPERIMENTS.

Platinum electrodes similar to those designed by Galaeotti were used. These were "platinized" with a solution of platinic chloride containing a trace of lead acetate. Since the muscle was in contact with the electrodes, there was danger of rubbing off some of the platinum black. I found that the black adhered more strongly if the electrodes were previously roughened by coating with platinum black and then heating in a flame.

The method of Kohlrausch was used to measure the conductivity. The smallest sized induction coil made for such experiments was fitted with a rheostat in the primary, and only just enough current was used as is required to work the interrupter. A second rheostat was inserted in the secondary, and the current could be reduced until it was not felt when passed through my tongue.

The muscles of the frog's thigh were used. They were placed between, and with the fibres parallel to, the electrodes, and the latter pressed together until the muscle bulged out on all sides. The elec-

⁵ DU BOIS REYMOND: Untersuchungen über tierische Electricität, 1849.

⁶ KODIS: This journal, 1901, v, p. 267.

⁷ GALAEOTTI: Zeitschrift für Biologie, 1902, n. F. xxv, p. 289, and 1903, xxvii, p. 65.

trodes were then fixed rigidly in position and the preparation placed in a moist, constant temperature chamber.

The conductivity of the muscle was measured with too little current to cause contraction. Then, by cutting out resistance in the secondary circuit, enough current was passed to throw the muscle into tetanus and a second reading made. In all cases the conductivity was greater during contraction.

It might be objected that the heating effect of this current would change the conductivity, but control experiments on liver tissue and the tissues of certain plants, in which no change in conductivity occurred, showed this not to be the case.

Since the muscle at all times entirely filled the space between the electrodes and extended out on all sides, a change in the form of the entire muscle would not appreciably alter the conductivity. However, it is possible that the change in form of the muscle fibres might slightly alter the conductivity, but it is improbable that it would account for the large differences observed.

If the change in conductivity were due to metabolic activity in the muscle, we would expect different results depending on whether the muscle was measured first in the stimulated or unstimulated condition, but no such difference was found. If the change in conductivity is due to chemical change, the latter must be completely and instantaneously reversible.

Experiments were made both in the spring and the autumn, and a long series of measurements were made on each muscle. The increase in conductivity was greatest in fresh preparations and decreased as the muscle became fatigued, varying from 28 to 6 per cent.

DISCUSSION OF RESULTS.

The increased conductivity of muscle during contraction may be interpreted as demonstrating the increase in permeability of some structures within the muscle to anions (since the muscle appears already permeable to certain cations). According to the membrane theory, this causes a reduction of the electrical polarization of these structures, thus causing increased surface tension and contraction.

In order that the contraction be due to increased surface tension of any structures, the latter must during relaxation be elongate in

the axis of the muscle. We have such a structure in the anisotropic segment of an ultimate fibril. It is interesting to note that Duesberg⁸ finds these segments to arise from fat or lipoid-containing bodies known as chondriosomes, and the presence of fat would account for the high surface tension between them and the sarcoplasm that is necessary for contraction.

Bernstein calculated the size of the structures that would be compatible with the force of contraction, and concluded that it must be smaller than any structures seen in histological preparations of muscle. He therefore postulated hypothetical ellipsoids as the elements in question.

It is possible that surface tension changes are aided by osmotic pressure,⁹ since the movements of plants are due to osmotic changes following changes in permeability. However, the small size of the muscle elements makes it impossible to apply botanical methods to it.

⁸ DUESBERG: *Archiv für Zellforschung*, 1910, iv, p. 602.

⁹ Cf. MEIGS: *This journal*, 1910, xxvi, p. 191.

THE UNIVERSITY PRESS, CAMBRIDGE, U.S.A.

THE OSMOTIC AND SURFACE TENSION PHENOMENA OF LIVING ELEMENTS AND THEIR PHYSIO- LOGICAL SIGNIFICANCE.¹

J. F. McCLENDON.

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PREFACE.

This paper formed the basis for two lectures given before the class in physiology at Woods Hole, July 7 and 8, 1911, although owing to limited time, some parts were omitted. Since then there has appeared a second edition of Höber's "Physikalische Chemie der Zelle und Gewebe," which reviews much of the literature considered in this paper. However, owing to an entirely different mode of presentation, it is hoped that the present treatment of the subject might be helpful to many general readers, some of whom would not read Höber's book.

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I am indebted to several persons for suggestions, especially to Dr. Ralph Lillie¹ and Professor B. M. Duggar.

I. INTRODUCTION.

The object of this paper is to bring the "vital" phenomena, as far as possible, within the scope of physics and chemistry, and not to elucidate physical and chemical processes. It should therefore be borne in mind that the osmotic phenomena of "dead" systems are not all satisfactorily explained.

The Vant Hoff-Arrhenius theory of osmosis concerns itself with the number of particles, molecules and ions, in solution, and is applicable to dilute solutions, in which the total volume of the dissolved particles is negligible. However, in more concentrated solutions, the volume of the dissolved particles is of the same importance as the volume of the molecules in gases, as expressed in Van der Waal's equation. Also the dissolved particles bind molecules of the solvent and so reduce the volume of the free solvent.

That the molecules and ions of a dissolved substance bind some molecules of the solvent, follows from the work of Jones and his collaborators.² Compare also the work of Pickering.³ Jones concludes that the larger the number of molecules of water of crystallization, the greater the hydrating power of a substance in aqueous solution. The number of molecules of water bound by one molecule of the solute usually increases with dilution up to a certain point (the boundary between concentrated and dilute solutions, beyond which there is no heat of dilution). The bond between ions and the solvent is also indicated by the phenomenon known as "electrical transference." If an electrolyte and a non-electrolyte be dissolved in water and an electric current passed through the solution, water will be carried along with the ions to the electrodes.

With these corrections, the Vant Hoff-Arrhenius theory accounts for osmotic pressure, but does not show why many substances exert no osmotic pressure, in other words, why no

¹ Cf. this journal, 1909, XVII., 188.

² "Hydrates in Aqueous Solution," Pub. No. 8, Carnegie Ins. Wash., 1907.

³ Whetam, "The Theory of Solution," 1902, Cambridge, p. 170.

membranes have been found that are impermeable to them. Overton supposed that the substance, in order to diffuse, must dissolve in the membrane. Kahlenberg and others consider a solution as a chemical combination between solute and solvent, and osmosis as a series of chemical reactions between the membrane and the two solutions, continuing until equilibrium is established. The essential points in the theory are: that the membrane is not a molecule sieve, but a substance with specific properties, and the chemical characters of the membrane and of the dissolved substances affect osmosis.

Willard Gibbs found that the more a solute lowers the surface tension of a solution, the more it tends to pass out of the solution, *i. e.*, by osmosis, or if this is prevented, to collect at the surface of the solution. This law has been extensively investigated and confirmed by I. Traube. For instance, in general, lipoid-soluble substances lower the surface tension of water and tend to diffuse out of it, whereas electrolytes slightly raise the surface tension of water and attract water from the adjacent phase. Osmosis may occur in opposite directions simultaneously. Gibbs and Traube state that the greatest osmotic flow is from the solution of lower surface tension to that of the higher, but this is not generally accepted. Osmosis consists of two distinct processes, from one solution to the membrane, and from the membrane to the second solution.

In case the membrane consists of two or more chemically different membranes placed one on another, osmosis consists of a series of steps; and Hamburger¹ made double membranes through which certain substances diffuse more rapidly in one direction than in the other.

Traube calls the bond between solute and solvent the "attraction pressure." In general, attraction pressure of ions increases with valence. The less the attraction pressure of the solute, the more it lowers the surface tension and tends to pass out of the solution. The presence of one solute lowers the attraction pressure of another in the same solution, and the greater the attraction pressure of a solute the more it lowers that of another. We might express this idea by saying that one substance takes

¹ *Biochem. Zeit.*, 1908, XI., 443.

part of the solvent away from the second and increases the concentration of the second substance. This may explain the effect of a harmless substance in increasing the toxicity of a poison. Schnierlen¹ observed that a solution of phenol below the threshold of toxicity for certain bacteria is rendered toxic by adding NaCl. Stockard showed that the toxicity of pure solutions of salts on fish eggs is increased by the addition of sugar, although the total osmotic pressure of the mixture is less than that of the normal medium.²

Just as Traube's precipitation membranes are absolutely impermeable to certain substances, so do living cells show this selective permeability. For instance, the vacuole fluid or cell sap of certain plant cells contains colored substances which do not diffuse into the protoplasm surrounding the vacuoles. If a cell be placed in a solution of the pigment, the protoplasm remains colorless. If the protoplasm be squeezed out of the cell into a solution of the pigment, it does not invariably become stained. However, if the cell is injured in certain ways, or dies from any cause, the pigment diffuses out of the vacuoles into the protoplasm and thence into the surrounding medium. We might conclude that the protoplasm in general is impermeable to the color, but at death it becomes permeable. On the other hand, Pfeffer³ gives evidence for the existence of a mechanical membrane on the surface of the cell and lining the vacuoles. De Vries⁴ placed cells into 10 per cent. KNO_3 solution colored with eosin. The plasma membrane and granular plasma died and stained long before any dye entered the vacuoles. However, the granular plasma may have absorbed all the dye, thus preventing its entrance for some time, without the necessity of any resistance of the vacuole membrane. Since protoplasm may be squeezed out in the form of droplets and still appears to be surrounded by membranes, Pfeffer concluded that the membrane was formed by the contact of the protoplasm with the medium

¹ *Arch. exp. Path.*, 1896, XXXVII., 84.

² However the NaCl in Schnierlen's and sugar in Stockard's experiment may have increased the permeability to the toxic substances, as discussed in later chapters.

³ "Pflanzenphysiologie."

⁴ *Jahrb. wiss. Bot.*, 1885, XVI., 465.

or with cell sap. He supposed these membranes to be the semipermeable parts of the cell, and that they became altered at death. Pfeffer called this membrane on the cell surface the "plasma membrane."

Whereas the nuclear membrane and certain vacuole membranes are semipermeable, these are lacking in erythrocytes, which are therefore good objects for testing the question whether the protoplasm in general, or merely its surface, is semipermeable. Höber¹ by two very ingenious but complicated methods, one based on dielectric capacity, determined the electric conductivity of the interior of the erythrocyte without rupture of the plasma membrane. Since the conductivity of the interior (about that of a .2 per cent. NaCl solution) was found to be many times greater than that of the erythrocyte as a whole, the membrane must be relatively impermeable to ions. There is much other, but less direct, evidence that the semipermeability resides in the plasma membrane, namely: the rapidity of change in permeability of certain cells, the sudden increase in permeability of a cell after swelling to a certain size (due presumably to rupture of the plasma membrane), the ease with which mild mechanical treatment increases the permeability, and the localization of electric polarization at the cell surface.

Quincke² supposed these membranes to be of a fatty nature. This idea was carried further by Overton, who considered the plasma membrane to be composed, not of neutral fats, but of substances of the class which are called "lipoids," which included non-saponifying ether soluble extracts of organs, *i. e.*, cholesterin, lecithin, cuorin, and cerebrin. He found³ that all basic dyes were easily absorbed by living cells, but not most of the sulphonic acid dyes. This corresponded to their solubility in melted cholesterin, or solutions of lecithin and cholesterin, or particles of lecithin, protagon or cerebrin. His argument is somewhat weakened, however, by the fact that cholesterin decomposes on melting, and that if lecithin is allowed to absorb water its solvent power changes.

¹ *Arch. f. d. ges. Physiol.*, 1910, CXXXIII., 237, and Eighth Internat. Physiol. Congress, Vienna, 1910.

² *Sitzber. d. Kon. Preuss. Akad. d. Wissensch. zu Berlin*, 1888, Bd. XXXIV.

³ *Jahrb. wiss. Bot.*, 1900, XXXIV., 669.

Many of Overton's critics do not distinguish between lipoids proper and a host of ether-soluble substances which are also called lipoids, and of the data which they present we will consider only that on lipoids proper. Ruhland¹ found that certain dyes stain plant cells but are not soluble in solutions of cholesterin (and vice versa). Robertson² observed that methyl green freed from methyl violet was insoluble in a nearly saturated solution of lecithin in benzol, whereas it stained living cells. Höber³ obtained Ruhland's results, when using certain animal cells, but found that certain nephric tubule cells absorb all dyes that are not suspension colloids.

Faure-Fremiet, Mayer and Schaeffer⁴ state that pure cholesterin does not stain with any dyes (contrary to Overton), malachite green (considered lipoid-insoluble by Ruhland and Höber) stains lecithin, and Bismarck brown (considered lipoid-insoluble by Ruhland) stains lecithin, cholesterin-oleate and cerebrin. A mere trace of free fatty acid greatly affects the behavior of lipoids toward stains.

Mathews⁵ considers the absorption of dyes by cells as a chemical process. Since basic dyes combine with albumin in alkaline solution, lipoids in the membrane are not necessary for the absorption of such dyes.

Traube objected to Overton's hypothesis on the ground that Overton's plasmolytic series is the same as found by Brown, who used the membrane of the barley grain,⁶ and the same as the series of the attraction pressures of the substances in water. But Traube admits in his later papers that the chemical character of the membrane affects osmosis.

We may conclude that, although the plasma membrane of some cells may be lipoid in character, this has not been proven, but, in general, it is more permeable the more the diffusing substance lowers the surface tension of water.

¹ *Jahrb. wiss. Bot.*, 1908, XLVI., 1, and *Ber. Deutsch. bot. Gesellsch.*, 1909, XXVI., 772.

² *Jour. Bio. Chem.*, 1908, LV., 1.

³ *Biochem. Zeit.*, 1909, XX., 55.

⁴ *Arch. d'Anat. Mic.*, 1910, XII., 19.

⁵ *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

⁶ But this is not true of all seed coats. Atkins, *Sci. Proc. Roy. Dublin Soc.*, XII., n. s., No. 4, p. 35, observed that the membranes of the bean seed are freely permeable, semipermeable plasma membranes arising only after germination.

Nathanson¹ supposed the plasma membrane to be a mosaic of lipoids and "protoplasm," but it is evident that if the lipoid portion is not continuous, it can not make the cell impermeable to any substance.

Czapek² states that lipoid solvents cause cytolysis when the surface tension of the solution is reduced to .68, and concludes from this that the plasma membrane contains glycerine tri-oleate since its emulsion reduces the surface tension of water to this figure.

The diffusion of water-soluble substances through swollen-plates, "gels" or "sols" of gelatine, varies inversely with the viscosity (Arrhenius). The great hysteresis of gelatine gels is taken advantage of to show that diffusion depends on the viscosity and not on the per cent. of gelatine, at the same temperature.³

The absorption of water by a gelatine plate increases its permeability, and the temperature and therefore the presence of substances which affect this swelling of gelatine affect its permeability. Impregnation of colloidal membranes with bile salts, alcohol, ether, acetone or sugar changes (usually increases) their permeability. The effects of substances on the rate of diffusion through gelatine plates, and on their swelling (viscosity) and melting point are not always quite parallel.⁴

In case the substance added to the membrane is removable, the change in permeability becomes reversible, which is true in regard to many of the substances mentioned above. Changes in non-living membranes are usually only partially reversible or are irreversible. Denaturalization of a colloid membrane by heat, heavy metals, or other coagulative agents which induce chemical changes in the membrane, or the addition of substances which cannot be removed, produce irreversible changes in permeability.

That the permeability of the membranes in living tissue is increased at death is proven by a host of observations. The electric conductivity increases enormously at death. Contained

¹ *Jahrb. wiss. Bot.*, 1903, XXXVIII., 284; 1904, XXXIV., 601, and XL., 403.

² *Ber. deutsch. bot. Gesell.*, 1910, 28, 480.

³ Zangger, Asher & Spiro's *Ergeb. der Physiol.*, 1908, VII., 99.

⁴ Zangger, *loc. cit.*

substances diffuse out, substances in the medium (fixing fluids, stains, etc.) diffuse in. There is a more general mixing of tissue substances. Enzymes come in contact with proteids and autolysis results.

Certain substances are known to increase the permeability of membranes in tissues of the body. Thus ether, chloroform, etc., increase the penetration of fixing fluids, and the exit of contained substances, and the mixing of tissue substances. In this way they increase autolysis.

II. OSMOTIC PHENOMENA IN PLANTS.

It is evident that water, salts, carbon dioxide and oxygen can, at least occasionally, penetrate plant cells, as otherwise no growth could occur. In case of the higher plants, the same is true of sugars and other bodies.¹ Janse² found that so much KNO_3 is absorbed by *Spirogyra* cells in 10 minutes, that it may be easily detected microchemically with diphenylamin-sulphuric acid.

Osterhout³ grew seeds of *Dianthus barbatus* in distilled water. The rate of growth during the several days of observation was normal. In nature, calcium oxalate crystals are found in the root hairs, but are not formed in the distilled water cultures, showing that the Ca comes from the medium. If placed in calcium solutions, crystals became large enough to see with the polarizing microscope in four hours, showing permeability to Ca.⁴

Nathanson⁵ found that nitrates and other substances entered the cell. Ruhland also observed penetration of salts.

Traube-Mengarini and Scala⁶ conclude that salts enter plant cells only through the partition walls. At these places there appears an "acid reaction" (bluing of methyl violet). They

¹ See Laurent in Livingstone, "The Role of Diffusion and Osmotic Pressure in Plants," 1903, p. 67.

² *Versl. en Medeel. der Koninkl. Akad. van afdeel. Naturs.*, 3. Reeks, IV. part, 1888, p. 333.

³ *Zeits. f. physik. Chem.*, 1909, LXX., 408.

⁴ But compare von Mayenberg, *Jahrb. f. wiss. Bot.*, XXXVI., 381, who found little penetration of salts into fungous hyphæ. And see Demoussy, *Comptes Rendus*, CXXXVII., 970.

⁵ *Jahrb. wiss. Bot.*, XXXVIII., 284; XXXIX., 601; XL., 403.

⁶ *Biochem. Zeit.*, 1909, XVII., 443.

interpret this as showing that the anion of the salt unites with an H ion of an amino group, forming a free acid, and the kation of the salt unites with the protoplasm. It appears to me that the basis of this conclusion is very slight.

Permeability may be investigated by a study of plasmolysis, which consists in the shrinkage of the surface protoplasm away from the cellulose cell wall, due to the osmotic pressure of the hypertonic solution of a dissolved substance which does not penetrate. A regaining of turgor by the cell while in the hypertonic solution indicates slow penetration of the substance. The plasmolytic method was originated by Nageli, who also noted that a shrinkage resembling plasmolysis but accompanied by outward diffusion of dissolved substances, occurs at death or severe injury to the cell.¹

The plant cell is surrounded by an elastic cell wall. The internal osmotic pressure may be divided into three resultants: that causing rounding up of the cell is called turgor, that resulting in stretching of the cell wall is sometimes distinguished as turgescence, and that resisting the surface tension of the cell, "central pressure."

The plasmolytic experiments of DeVries² and others³ are interpreted by them as indicating a selective impermeability of the plasma membrane to neutral salts.

In the plasmolytic experiments of Overton⁴ all salts plasmolyzed permanently. Non-electrolytes fell in four groups, thus: Cane sugar, dextrose, manit, glycocoll > urea, glucerin > ethylene-alcohol, acetamid > methyl-alcohol, acetonitril, ethyl-alcohol, phenol, aniline, isobutyl-alcohol, isoamyl-alcohol, methyl acetate, ethyl acetate, butyl aldehyde, acetone, acetaldoxim. Diffusion of substances of homologous series increased with molecular weight.

Overton ascertained the permeability of plant cells to alkaloids

¹ "Pflanzenphysiol. Untersuchungen," 1885.

² *Zeit. physikal. Chem.*, 1888, II., 415; 1889, III., 103.

³ Cf. Livingstone, "The Rôle of Diffusion and Osmotic Pressure in Plants," Chicago, 1903; Janse, *Bot. Centlb.*, 1887, XXXII., 21; Duggar, *Trans. Acad. Sc. St. Louis*, 1906, XVI., 473.

⁴ *Vierteljahrschrift der Naturforschers. Gesell. in Zurich*, XLIV., 88; *Jahr. wiss. Bot.*, 1900, XXXIV., 669.

by their precipitation of the tannic acid in the cell sap. Most alkaloids penetrate rapidly, but only in the form of the free (undissociated) base produced by hydrolysis. Hence the penetration (precipitation and toxic effect) may be prevented by adding a little acid to the medium.

Pfeffer had shown that methylene blue is precipitated by tannic acid in the cell sap of certain plants.

Some discussion has arisen as to whether the mechanism of the entrance of dyes into plant cells is similar to that of alkaloids. Overton showed that lipoid soluble basic dyes penetrate easily. He at first supposed that only the free color base (undissociated) is able to penetrate the cell.¹ Overton found, however, that triphenylmethane and chinonimid dyes disprove his assumption, showing that it is at least not general. This question was taken up again by Harvey² who found that neutral red or methylene blue, which stain *Elodea* leaves in tap water, do not do so if just enough acid be added to the water to prevent any free color base from forming.

He observed that, although these dyes are not precipitated in the cell sap of this plant, they become more concentrated in the cell sap than in the medium. Neutral red is bright red in the cell sap, indicating that the reaction is acid (no free color base is present). He supposes that the absence of any of the dye in the form of the free color base prevents it from diffusing out of the cell, hence it becomes more concentrated within than without.

In using the plasmolytic method, if a cell does not recover from plasmolysis in a solution of a salt, it is said to be impermeable to that salt. However, the cell may recover, but may be killed by penetration of the salt, and shrink again. It is possible that Overton and others failed in some cases to note this transient recovery. Contrary to Overton, Osterhout³ found *Spirogyra* permeable to alkali-salts and alkaline earth salts, but more

¹ In this connection it is interesting to note that Robertson observed that free color bases, and to a less extent free color acids, are much more soluble in fats than are their salts. This is what we should expect, since the salts dissociate in water, and ions are insoluble in fats.

² *Science*, 1910, n.s., XXXII., 565.

³ *Science*, 1911, n. s., XXXIV., 187; XXXV., 112.

easily to Na than to Ca. It is plasmolyzed by $.2M$ CaCl_2 and not by the isosmotic $.29M$ NaCl but by $.38M$ NaCl . $.195M$ CaCl_2 and $.375M$ NaCl just failed to plasmolyze. On mixing 100 c.c. $.375M$ NaCl with 10 c.c. $.195M$ CaCl_2 , thus decreasing the osmotic pressure of the former, marked plasmolysis occurred. This indicates that Ca decreases the permeability to Na.¹ From further work by the same author, not yet published, it appears that Na increases and Ca decreases the permeability of certain marine plants. Also Fluri² obtained increase in permeability by salts of aluminium, yttrium and lanthanum.

DeVries plasmolyzed cells of *Tradescantia*, containing blue cell sap, with 4 per cent. KNO_3 solution, then added nitric acid until the color changed to red. The acid made the cells permeable to KNO_3 for they regained their turgor and finally burst. This explains the easy penetration of acids into cells. Pfeffer³ found that if red beet cells, petals of *Pulmonaria*, stamen hairs of *Tradescantia* and other anthocyan-containing cells are placed in extremely dilute HCl or H_2SO_4 , they suddenly turn red, indicating immediate penetration of the acid. If allowed to remain but a short time, the cells are not killed, and the color change is reversed on returning the tissues to acid-free water.

I have repeated these experiments, using cells of red beet, red cabbage and red nectar glands of *Vicia faba*, and find that mineral acids penetrate, but that (the lipoid soluble) acetic acid penetrates much more rapidly and also more easily alters the plasma membrane, causing pigment to diffuse out, if not cautiously applied. Alkalis also penetrate, but (the lipoid soluble) ammonia penetrates much more rapidly than the others. Ammonia does not so easily increase the permeability to the pigment as does acetic acid.

Ruhland⁴ after staining root hairs of *Trianea*, etc., with the indicators, methyl orange and neutral red, found that mineral acids as well as lipoid soluble acids penetrated.

¹ The work of Kearney, Report 71, U. S. Dept. of Agriculture, indicates that Ca prevents the plasmolytic and toxic effect of Mg, but this is "false plasmolysis" following death.

² *Flora*, 1908, XCIX., 81.

³ "Osmotische Untersuchungen," Leipzig, 1877, p. 135.

⁴ *Jahrb. wiss. Bot.*, 1908, XLVI., 1.

One defect in the plasmolytic method is the fact that the cellulose cell wall, if not very thick, is elastic, and a slightly hypertonic solution may cause the cell to decrease in volume without pressing the protoplasm away from the cell wall. This source of error may be eliminated by substituting calculations of the volume of the cells (as necessary for animal cells) for observations on plasmolysis.

It is well known that movement, and in many cases increase in size of plants is due to changes in turgor of the cells. If we exclude the turgor changes in aerial plants produced by variations in the ratio of the water supply to the transpiration, turgor changes may be due to changes in the osmotic pressure of the external medium, or of the cell sap (due to metabolic changes) or to changes in the permeability of the plasma membrane. Lepeschkin¹ has confirmed Pfeffer in showing that changes in permeability of stipule cells accompany (or immediately precede) changes in turgor. By chemical analysis of the medium he has shown that an outward diffusion of dissolved substances, from the cells, accompanies loss of turgor, and by plasmolytic experiments, that the permeability to certain substances increases.

It is interesting to note the force that may be exerted by such changes in turgor. From measurements of the pull of a stamen hair of *Cynara scolymus* or *Centaurea jacea* on loss of turgor following stimulation, it seems not improbable that the change in turgor amounts to 2–4 atmospheres (Höber). This also indicates the strength of the cell wall necessary to prevent rupture of the plasma membrane. The osmotic pressure of the juices pressed out of plants varies from 3.5–9 atmospheres.² The pressing out of the juices causes an error due to chemical changes; on the other hand, in taking the freezing point or pieces of plant tissues, an error arises from lowering of the freezing point by the walls of the capillary spaces. Müller-Thurgau³ found the Δ (corrected freezing point lowering) of plant tissues = .8–3.1°. Many plants respond to light by definite movements, produced

¹ *Ber. deutsch. bot. Gesell.*, XXVI. (a), 725.

² DeVries, *Pringsheime Jahrbucher wiss. Bot.*, 1884, XIV., 427; Pantanelli, *ibid.*, 1904, XL., 303.

³ *Landwirtschaftl. Jahrb.*, 1886, XV., 490.

by turgor changes in certain of their cells. Trondle¹ found that light produced changes in permeability of these cells.

Changes in permeability may not only affect the turgor, but also the assimilation and excretion, and consequently the metabolism and growth of the cells. Chapin² observed that CO₂ in certain doses is a stimulant to the growth, not only of green plants but also of moulds. As only a few saprophytes can decompose CO₂, it is not probable that its effect is nutritive. A similar stimulating action of ether and various salts, even such toxic ones as those of zinc, was previously known. These salts probably stimulate without penetrating the cells, since Zn, for instance, is not a constituent of protoplasm.³ This leads one to suppose that the initial effect of all of these substances is on the surface, changing the permeability of the cells.

Wächter⁴ found that potassium decreases the permeability of onion cells. Sugar diffused out of sections of *Allium cepa* placed in distilled water or hypotonic sugar solutions, but a trace of potassium salt entirely prohibited the diffusion. When the K was removed the diffusion recommenced.

Czapek⁵ determined increase in permeability by the exosmosis of tannin in cells of *Echeveria* leaves. Various monovalent alcohols and ketones, ether, ethyl urethan, di and tri acetin, Na-oleate, oleic acid, lecithin and cholesterin all just caused exosmosis of tannin in concentrations (aqueous solutions) which had a surface tension of about 0.68. It would appear therefore that these substances, chiefly of the class of indifferent narcotics, alter the cells if they diffuse into them, or diffuse into certain structures such as the cell lipoids or the plasma membrane. It seems more reasonable to suppose that the plasma membrane is the structure affected, and the more the substance lowers the surface tension of water, the more it diffuses into the plasma membrane. When this membrane is altered, it allows escape of tannin. Some substances such as chloral hydrate are effective

¹ *Jahrb. f. wiss. Bot.*, 1910, XLVIII., 171.

² *Flora*, 1902, XC., 348.

³ Cf. Loeb, "Dynamics of Living Matter," pp. 73, 74.

⁴ *Jahrb. wiss. Bot.*, 1905, XLI., 165.

⁵ "Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen," Jena, G. Fischer, 1911.

in less concentration, and probably affect the cell chemically as well as physically.

Mineral acids caused exosmosis of tannin when the concentration just exceeded 1/6,400 normal, and the effect is probably due to H ions. At this same concentration Kahlenberg and True¹ found the growth of seedlings of *Lupinus albus* to cease. It appears, therefore, that this cessation of growth is due to increased permeability, causing decreased turgor of the cells.

Changes in permeability may also affect secretion (excretion). The addition or formation of alcohol or acetates causes yeast and other fungi to secrete (excrete) for a short time, various substances, especially enzymes which do not come out in a culture medium lacking the reagent.² It appears that the alcohol or acetates increase the permeability of the fungi to these substances.

My own experiments³ indicate that pure $MgCl_2$ solutions increase the permeability of yeast. A certain per cent. of yeast and dextrose in .3 molecular $MgCl_2$ eliminated CO_2 more rapidly than .5M NaCl or .325M $CaCl_2$, all which have about the same freezing points. Also, the CO_2 elimination was more rapid in the magnesium solution than in a solution of the same concentration of $MgCl_2$ with either of the other salts in addition, or in a solution containing NaCl and $CaCl_2$ in the same concentrations as in their respective pure solutions, or in a solution of all three salts, or in tap or distilled water. In order to determine whether the magnesium entered the cells I took two equal masses of compressed yeast and agitated one in H_2O and the other in a molecular solution of $MgCl_2$ for 5 hours, then washed each rapidly in H_2O by means of the centrifuge. The ash of the magnesium culture = .048 gram, that of the control = .0466 gram. Evidently the Mg did not enter the yeast to any great extent, and probably acted on the surface, increasing the permeability.

Ewart⁴ observed that after placing plant tissue in 2 per cent. HCl and washing in water its electric conductivity (ionic permeability) was increased. If one portion of the plant is stimulated, the stimulus may be transmitted to other portions. In

¹ Kahlenberg and True, *Botanical Gazette*, 1896, XXII., p. 81.

² Zangger, "Asher and Spiro's *Ergeb. d. Physiol.*," 1908, VII., 144.

³ McClendon, *Am. Jour. Physiol.*, 1910, XXVII., p. 265.

⁴ "Protoplasmic Streaming in Plants," Oxford, 1903, p. 96.

this way increase in electric conductivity was produced by stimulation of a point outside the path of the current.

Whereas many plants are very sensitive to sudden and extreme changes in osmotic pressure, Osterhout¹ found that certain marine algæ thrived when subjected daily to a change from fresh water, to sea water evaporated down until it crystallized out, and vice versa. He does not state whether these algæ survive extreme plasmolysis, or whether they are so easily permeable to salts as not to be plasmolyzed by the saturated sea water or burst by the fresh water.

For regulation to slight changes in the osmotic pressure of the medium, a change in size of the cell altering the turgescence, or tension of the cell wall, is sufficient.

If *Tradescantia* cells are placed in a hypotonic solution, they begin to swell. But soon crystals of calcium oxalate are formed in the cell sap, and in this way the turgor, due chiefly to oxalic acid, is reduced.² It would be interesting to know what is the source of the Ca. Was it previously in combination with proteids?

The accommodation to a hypertonic medium takes place, according to van Rysselberghe, partly through absorption of substances of the medium and partly through metabolic production of osmotic substances, chiefly the transformation of starch into oxalic acid.³

III. BIO-ELECTRICAL PHENOMENA.

I. *In Plants.*

Change in permeability of the plasma membrane to ions would necessarily cause electrical change due to its influence on the migration of ions. These electrical changes actually occur, and may be easily studied.

Stimulation or wounding in plants is accompanied by an electronegative variation of the affected surface. This negative region spreads in all directions over the surface, but the rate of

¹ Univ. of Cal. Pub., Bot., 1906, II., 227.

² Van Rysselberghe, Mém. d. l'Acad. royale de Belgique, 1899, LVIII., 1.

³ Compare von Mayenberg *Jahrb. f. wiss. Bot.*, XXXVI., 381.

propagation¹ is much slower than the similar process in muscle or nerve.²

Pfeffer³ supposed that the plasma membrane is normally permeable to ions of only one sign. Since the normal cell surface is positive in relation to the cell interior (cut surface) we may conclude that the plasma membrane is normally more permeable to kations (less permeable to anions). Just as the negative variation of wounding is due to the removal or rupture of the plasma membrane, so the negative variation of stimulation would, on the membrane hypothesis, be due to increase in permeability of the plasma membrane to the confined anions.

An alternative hypothesis is that these electrical changes result from changes in metabolic activity. The production of an electrolyte whose anion and kation have very different speeds of migration (such as an acid or alkali) would cause electrical changes. But how are we to account for changes in metabolic activity? There exists varied evidence for changes in permeability, and it is simpler to assume that changes in metabolic activity and electrical changes are both the result of changes in permeability.

Kunkel⁴ tried to explain the vital electrical phenomena as the result of the movement of fluids in the vessels of the tissues, but bio-electrical changes may occur without such movement of fluids (Burdon-Sanderson).

Kunkel observed in 1882⁵ that the movement of the leaf of *Mimosa pudica* is accompanied by an "action current," or negative variation of one surface of the pulvinus. Similar results on *Dionaea* leaves were obtained by Munk⁶ and specially studied by Burdon-Sanderson.⁷ It was stated above that Lepeschkin had shown that the turgor changes in plants were accompanied or immediately preceded by changes in permeability to certain substances. The electrical phenomena suggest that the turgor

¹ Which is in *mimosa* 600–1,000 times as fast as the geotropic impulse in a root.

² Fitting, "Asher and Spiro's *Ergeb. d. Physiol.*," 1906, V., 155.

³ "Pflanzenphysiologie."

⁴ *Arch. f. d. ges. Physiol.*, 1881, XXV., 342.

⁵ See Winterstein's "Handbuch der vergleichenden Physiologie," III. (2), 2, p. 214.

⁶ *Arch. f. Anat. u. Physiol.*, 1876, XXX., 167.

⁷ *Proc. Roy. Soc. London*, 1877, XXV., 441; *Philos. Trans.*, 1888, CLXXIX., 417.

change is accompanied (or immediately preceded) by increase in permeability of the plasma membrane to anions. Burdon-Sanderson states that, whereas the movement resulting from turgor change begins 2.5 seconds after stimulation, the negative variation reaches its maximum 1 second after stimulation. This may be due to the mechanical inertia, or the time required for the diffusion of substances.

It was stated in the preceding chapter that light changes the permeability of the plasma membrane, and Waller¹ found corresponding electrical changes due to light, but not always in the same direction in different plants. This inconstancy in direction is probably due to the fact that light not only influences the permeability, but also the assimilation, and changes in assimilation produce electric changes. This is supported by the fact that Querton² found that assimilation as well as electric change is most affected by the longer light rays.

2. *In Muscle and Nerve.*³

Ostwald⁴ proposed the hypothesis that the electric phenomena of muscle, nerve and the electric organs of fish (which may reach several hundred volts) are produced with the aid of semipermeable membranes. The alternative theory of Hermann, which would account for the current of injury by assuming the production of some electrolyte (alkali?) in the wounded region, whose anions and kations have very different speeds, seems less probably to be the correct one.

According to the "membrane theory," the muscle or nerve element is surrounded by a semipermeable membrane allowing easier passage to kations than to anions. The kations passing through the membrane are held back by the negative field produced by the confined anions, but owing to their kinetic energy, the kations pass out far enough to give the outside of the cell surface a positive charge. Therefore any portion of the surface that is made freely permeable to anions becomes electronegative

¹ *Jour. of Physiol.*, 1899-'00, XXV., 18.

² "Contribution à l'étude du mode de la production de l'électricité dans etres vivantes," *Travaux de l'Institut Solvay*, 1902, V.

³ Cf. R. Lillie, *Amer. Jour. Physiol.*, 1911, XXVIII., 197.

⁴ *Zeit. physik. Chem.*, 1890, VI., 71.

in relation to the remainder of the surface. This negative variation may be produced by artificially removing or altering a portion of the membrane (producing the current of injury) or as the result of normal stimulation, making it permeable to anions (action current).

Bernstein resorted to mathematical proof of this hypothesis. We will not here go into details, but the gist of the matter is that if the process were as we have imagined it, the electromotive force of the current of injury, or action current, should be proportional to the absolute temperature. He found this to be true for temperatures between 0° and 18° , but between 18° and 32° the E.M.F. was found to be too small. The muscle was not permanently injured by exposure to the higher temperatures for the length of time necessary for the experiments. Bernstein explained this discrepancy by the further assumption that at the higher temperatures the plasma membrane became slightly more permeable to anions.¹

Since the muscle contains a higher per cent. of potassium than the blood plasma or lymph, it might be supposed that K ions passed outward through the plasma membrane and gave the surface of the muscle element the positive charge. But if this were the case, the current of injury should be reversed by placing the muscle in a solution containing potassium in greater concentration than in the muscle. This reversal, however, was shown by Höber not to occur. Since lactic and carbonic acids are produced by muscle and diffuse out in increased amount on contraction, one might suppose H ions to give the muscle surface the positive charge. This is only a guess (and a poor one, since undissociated molecules of CO_2 and lactic acid are lipid-soluble) but may be convenient until some better one is proposed. Perhaps the carbonic acid combines with amphoteric proteids, which

¹ This is similar to the conclusion reached by Biataszewicz, *Bull. d. l'Acad. d' Sc. d. Cracovie, Sc. Math. e. Nat.*, Oct., 1908, p. 783, in regard to the unfertilized frog's egg. In order to explain his observation that the rate of swelling in tap water increased 5 times for every 10° rise in temperature, he assumed that heat increased the permeability to H_2O . This would seem to be the simplest explanation, provided the swelling were not due to chemical production of osmotic substances: and since the Δ of the ripe ovarian egg is $.48^{\circ}$ but is reduced to $.045^{\circ}$ after oviposition, *Biochem. Zeit.*, 1909, XXII., 390, much if not all of the swelling is probably due to the initial osmotic pressure of the egg interior.

then set free H^+ and HCO_3^- ions, thus increasing the ionization and therefore reducing the number of undissociated molecules, which can escape.¹

Since Osterhout showed that certain electrolytes may alter the permeability of cells, we might expect to find, on the membrane hypothesis, an effect of salts on the electric polarization of muscle. Höber² observed that a portion of the surface of a muscle treated with certain salts, KCl for instance, becomes electro-negative (more permeable to anions) whereas a portion treated with NaI or LiCl becomes positive (still less permeable to anions than is the normal unstimulated muscle). The order of effectiveness of the ions is as follows: $Li < Na < Cs < NH_4 < Rb < K$ and $CNS < NO_3 < I < Br < Cl < \text{valerianate, butyrate, propionate, acetate, formate} < SO_4, \text{tartrate}$. Similar ionic series were found by Overton, R. Lillie, Schwartz, Mathews, Grützner, Höber, and Mayer in the effect of salts on the functional activity of muscle, nerve and cilia, but the exact relation of these phenomena to permeability is not understood in every case. Pure solutions of salts of alkali metals may "inhibit" muscle by *increasing* permeability, but salts of alkali earth metals are said to "inhibit" by *decreasing* permeability. . . Mayer says that the effect of salts on cilia is the reverse of that of muscle, but the relation of this to permeability is not known. Since ions affect the aggregation state of hydrophile colloids in the same or exactly reversed order, and the kation series is found in no other known physico-chemical phenomena, it might be supposed that the semipermeable membranes of muscle are colloidal.

It seems probable that sugar solutions inhibit the activity of muscle by increasing the permeability, but since sugar is not an electrolyte this question cannot be tested by electric methods.

A negative variation of muscle may also be produced by the so-called "hæmolytic" substances, but is irreversible, whereas that produced by salts may be reversible. In this connection it

¹ Roaf, *Q. J. Exper. Physiol.*, 1910, III., 171, supposed the anion to be protein; however it has not been shown that proteids, or even amino acids diffuse out on stimulation. I do not see that the speculation of Galeotti, *Zeit. f. Allgem. Physiol.*, 1907, VI., 99, is at all explanatory.

² *Loc. cit.* and *Pflüger's Arch.*, 1910, CXXXIV., 311.

is interesting to note that Overton¹ found the permeability of muscle to be similar to that of plant cells.

It might appear to the reader that the membrane theory is merely wild speculation. What proof have we that on injury or during contraction the muscle is more permeable to any ion?

DuBois Reymond² and Hermann³ explained the fact that living muscle has a greater electric resistance than dead muscle on the hypothesis that the resistance of living muscle is due to the presence of membranes, which become more permeable at death. They demonstrated the resistance of muscle tissue to the passage of ions by the fact that electric polarization occurs in muscle tissue on the passage of an electric current. It seems to me that Kodis⁴ and Galeotti⁵ take a step backward, in attributing the decreased resistance of dead muscle to the liberation of ions. Galeotti tried to support his view by determinations of the freezing points of the living and dead muscle, but found on the contrary that the change in electric conductivity of the muscle did not correspond to the change in the osmotic pressure.

Du Bois Reymond⁶ observed that the electric conductivity of muscle changes on (during?) contraction and Galeotti⁷ found it to be greater on strong contraction than on weak contraction, and least on fatigue-exhaustion or cold-anæsthesia. However, the duration of a contraction is momentary (about 1/5 second for frog's muscle) and it is not clear that these investigators measured the conductivity accurately during such a brief period, in fact they probably measured it after contraction. Therefore I decided to repeat these experiments, using a method by which I could measure the conductivity during the actual contraction period, as well as in the unstimulated condition.⁸

¹ *Pflüger's Arch.*, 1902, XCII., 115.

² "Untersuchungen über thierische Electricität," 1849.

³ *Pflüger's Arch.*, 1872, V., 223, VI., 313.

⁴ *Am. Jour. Physiol.*, 1901, V., 267.

⁵ *Zeit. f. Biol.*, n. f., 1902, XXV., 289; 1903, XXVII., 65.

⁶ *Loc. cit.*

⁷ *Loc. cit.*

⁸ McClendon, *American Journal of Physiology*, 1912, XXIX., 302.

Experimental.

Platinum electrodes, platinized with platinic chloride containing a little lead acetate, and of a form similar to those designed by Galeotti, were used. Galeotti stimulated the muscle through the same electrodes used in measuring the electric conductivity, by switching on a different electric current. Though it were possible to throw a switch quickly enough to have the current for measurement of conductivity pass through the muscle during contraction, it would be necessary to use a string galvanometer to take the reading, and this method would probably not be very accurate. A more accurate method is that of Kohlrausch, in which a rapidly alternating current reduces polarization at the electrodes and in the tissue, but it is necessary to throw the muscle into tetanus in order to have time for the reading. I accomplished this by using the same current for stimulation and measurement of conductivity. A very small induction coil was fitted with a rheostat in the primary. Another rheostat in the secondary could be thrown out of the circuit by a switch. By adjusting the rheostats, a current strong enough to be distinctly heard in the telephone, yet too weak to stimulate the muscle, was obtained. By switching the resistance out of the secondary circuit, the current could immediately be increased so as to throw the muscle into tetanus. Since the Wheatstone bridge was used, the difference in current strengths had no direct effect on the readings. The conductivity increased from 6 to 28 per cent. (being usually about 15 per cent.) on stimulation.

We have, then, evidence for the increase in permeability of muscle to ions during contraction, but what relation has this to the mechanism of the contractile process? It has been suggested by D'Arsonval, Quincke, Imbert, Bernstein, Galeotti and others that the increased permeability to ions causes a disappearance of the normal electrical polarization of the elements, whose surface tension consequently increases, causing them to round up (shorten). But what are the elements concerned? It would be confusing to assume them to be the fibers, as then the function of the complicated internal structure would be unexplained. They are probably not the sarcous elements (portions of fiber between 2 Z-lines) as the rounding up of these ele-

ments would elongate the muscle. And even though contraction were produced by inequality in surface tension, as assumed by Macallum¹ the total surface change would be so small as not to account for the energy liberated in contraction. In order to avoid this last difficulty Bernstein made use of hypothetical ellipsoids. These were surrounded by elastic material to account for elongation of the muscle.²

The great differences of potential (several hundred volts) that may be produced by the electric organs of fish, is achieved by the arrangement of the modified muscle plates in series. All of the plates have the nerve termination on the same side. On stimulation of the nerve, each plate becomes negative, first on the nerve termination side, and thus the negative side of one plate touches the positive side of the next plate. In this way the direction of the current may be determined by studying the anatomy of the innervation. This rule, discovered by Pacini, finds an exception only in *Malopterurus*, whose electric organ is supposed by Fritsch to be derived, not from muscle but from skin glands.

The electric fish are *relatively* immune to electric currents passed through the medium. This is not merely an apparent immunity due to the fish being out of the path of the current, or the current being short circuited by sea water (in case of marine fish). I have received severe shocks from a torpedo that was entirely submerged in sea water.

3. *Amœboid Movement.*³

The normal unstimulated surface of plant and animal tissues is electro-positive in relation to the cut or injured surface of the cells. We have given reasons for assuming that this indicates greater permeability of the plasma membrane to kations than to anions, the latter accumulating in the cell interior, gives it a negative charge.

There are two reasons for believing that this is true also of the *Amœba*:

¹ *Science*, n. s., 1910, XXXII., 822.

² Meigs., *Am. Jour. Physiol.*, 1910, XXVI., 191, supposes the rounding up of muscle elements due to increased turgor.

³ McClendon, *Arch. f. d. ges. Physiol.*, 1911, CXL., 271.

1. If a weak electric current is passed through water in which an *Amæba* is suspended, it is carried passively toward the anode, indicating that it has a negative charge. This charge may be due to confined anions.

2. If a stronger electric current is passed through an *Amæba*, it begins to disintegrate first at that surface nearest the anode. The disintegration is probably due to the accumulation of ions retarded by the plasma membrane. The ions in the medium are free to pass around the *Amæba*, but the contained ions must pass the plasma membrane in order to migrate to the electrodes. Since the disintegration is toward the anode, it is probably due to anions which cannot get out of the *Amæba*. Since no corresponding disintegration begins toward the kathode, the plasma membrane is probably more permeable to kations.

The surface tension of the *Amæba* is very low, and apparently increases on strong stimulation (indicated by rounding up of the *Amæba*). We saw that stimulation in plant and muscle cells caused increased permeability to ions, and consequently disappearance of the normal electrical polarization, and thereby causing increased surface tension. We might conclude therefore that the low surface tension of the *Amæba* is caused by electric polarization, due to the production of some metabolic electrolyte whose anions cannot escape; and that strong stimulation causes increased permeability and hence disappearance of the electrical polarization.

This would explain all negative tropisms of the *Amæba*. The surface tension of the portion most strongly stimulated is increased, and the *Amæba* flows away from the stimulus.

In order to explain positive tropisms we would have to make another assumption. If the stimulus did not act directly on the plasma membrane, but penetrated the *Amæba* and acted on the protoplasm, and increased the production of the metabolic product producing polarization of the plasma membrane, it would thereby decrease the surface tension. The local decrease in surface tension would cause the *Amæba* to flow toward the source of the stimulus, just as the quicksilver drop in dilute HNO_3 flows toward potassium bichromate in Bernstein's experiment.

All stimuli producing positive tropism would then have to penetrate to a greater or less distance into the *Amæba*. But the same stimulus thus acting on the interior might, in greater intensity, affect also the plasma membrane, increasing its permeability and changing the positive to negative tropism. Such a change of the sign of tropism has been observed.

Soap lowers the surface tension of fats and lipoids, and Quincke, Bütschli, Loeb, Robertson and others supposed that lowering of the surface tension of living cells might be due to soap. However, I found that soap always causes negative tropism in *Amæba*, probably because it increases the permeability of the plasma membrane.

4. *The Propagation of the Bio-electric Changes.*

On the hypothesis, that the electric phenomena in muscle and nerve, as well as other animal and also plant tissues, is due to change in permeability to ions, we might hope to explain the wave-like propagation of these changes. Since extraneous electric currents "stimulate" all tissues (presumably by increasing permeability) thus causing them to produce additional electric phenomena, it seems natural that these latter would be self-propagating. It is probably the negative variation of nerve which stimulates the muscle, and the negative variation of the portion of the muscle fiber adjoining the nerve ending, which stimulates the adjacent portions of the muscle. Nernst found mathematical proof that electric stimulation is due to change in ionic concentration at the semipermeable membranes.

I have found evidence that the negative variation (current of injury) in plants, may strongly affect adjacent cells. If an electric current of suitable density is passed through plant or animal tissue, negatively charged colloids in the protoplasm migrate toward the anode. I have observed this movement in living cells, and the resulting displaced bodies in histological sections. In certain cases there may be some doubt whether the colloids moved toward the anode, or water toward the kathode, but in others, easily distinguishable bodies such as chromatin granules or threads moved toward the anode.

If the tip of a root be cut off we observe a negative variation

of the cut surface. This produces an electric current through the medium and surrounding tissue. The fact that the current actually passes through adjacent cells is shown by a displacement of their contained colloids, identical in appearance with the displacement produced by the currents used in the above experiments. Nêmec¹ apparently observed these changes but did not correctly describe or interpret them.

The fact that an electric current on increase (make) stimulates muscle at the kathode, and the fact that the muscle surface is normally positive in relation to the interior (cut surface), probably indicates that stimulation is produced by a rapid depolarization of the muscle surface.

If this reasoning be applied to an individual contractile element, we may assume that the current causes kations to leave the outer surface of the membrane, and other kations to be attracted toward the inner side of the membrane, and thus the polarization disappears or may even be reversed. Just how this causes an increase in permeability of the membrane is a matter which we will leave to the future for discussion.

It has been supposed that the stimulated region acts as kathode to the adjacent portions, and these in turn act as kathodes to the next portions and so the stimulus is propagated.

Stimulation of a part of the surface, causing it to become more permeable to ions, depolarizes the adjacent parts of the surface owing to the fact that confined anions migrate through the permeable region and neutralize the charges of the kations on adjacent parts of the impermeable region (see Fig. 1). For this reason the increase in permeability is propagated.

This explanation of the phenomenon in a single element holds for a tissue made up of many elements provided these are in contact, as illustrated by the accompanying Fig. 2. This is probably the mechanism of propagation of the negative variation (and "stimulus") in many plant and animal tissues.

This mechanism accounts for the movement of the negative variation over a surface. But it may be possible for this electric change to jump from one element, to another not touching it. The observations on the current of injury, cited above, show that

¹ "Reizleitung u. d. reizleitenden Strukturen b. d. Pflanzen," Jena, 1901.

increased permeability of part of a tissue surface, may cause electric currents to flow through cells some distance from the wound. These currents probably stimulate the cells through which they pass, which in turn become permeable and produce electric currents. This explains the propagation of stimuli

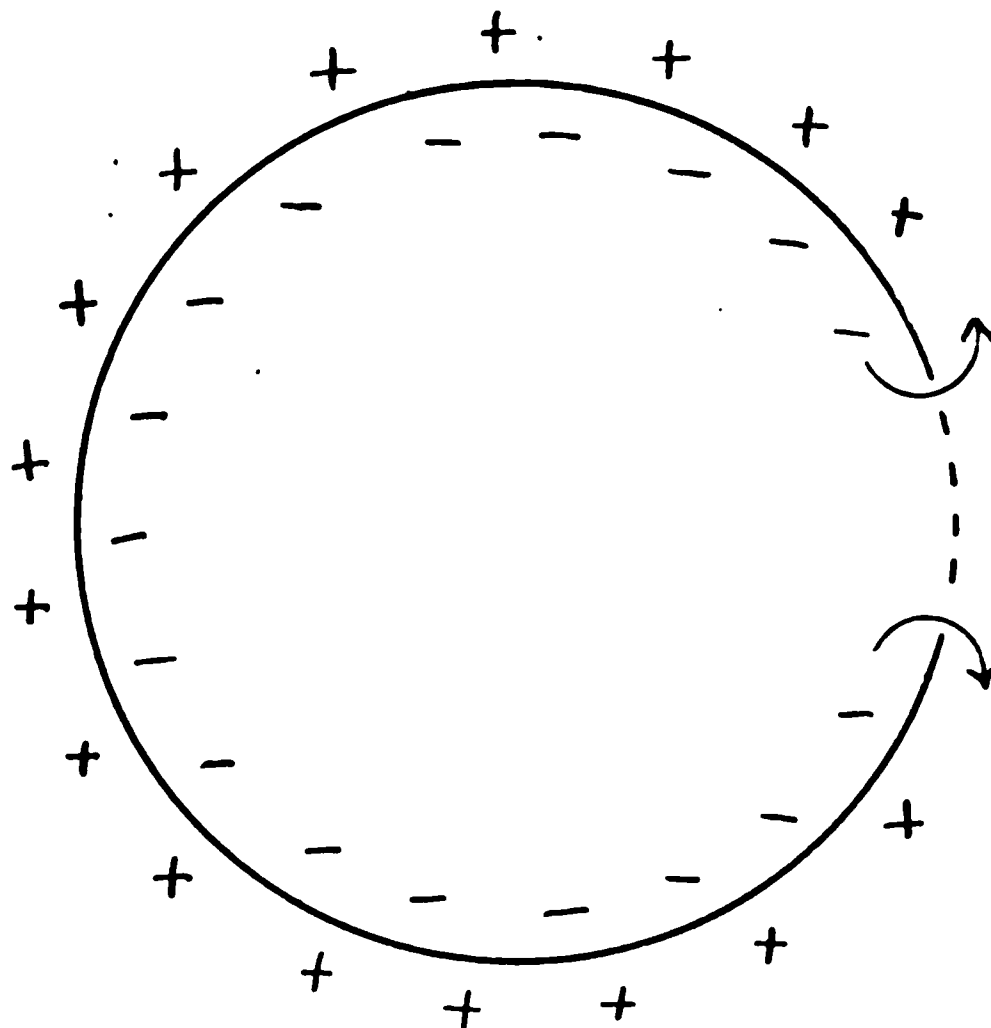


FIG. 1.

Anions represented by minus sign, kations represented by plus sign. Arrows denote the direction of migration of ions. The large circle represents the plasma membrane, the dotted line denoting the permeable and the continuous line, the impermeable portion.

through loose tissues, and the structural changes, as observed by Némec.

The rate of propagation of the "wound stimulus" is very slow, whereas that of propagation of the "stimulus" (negative variation) in sensitive plants is more rapid, and that of the nerve impulse still more rapid. We have not, however, sufficient data to show whether this is a mathematical objection to the hypothesis.

The streaming movements in plants may be stopped by a strong stimulus or "shock." This stimulus is usually propagated in one or more directions. Ewart¹ states that the rate of propagation at 18° in a single elongated cell of *Nitella* is 1-20 mm.

¹ *Loc. cit.*

per sec., but where it has to pass cell walls .001-.03 mm. per sec. However, the stoppage of the streaming was his criterion of the presence of the stimulus, and probably the banking of the stream

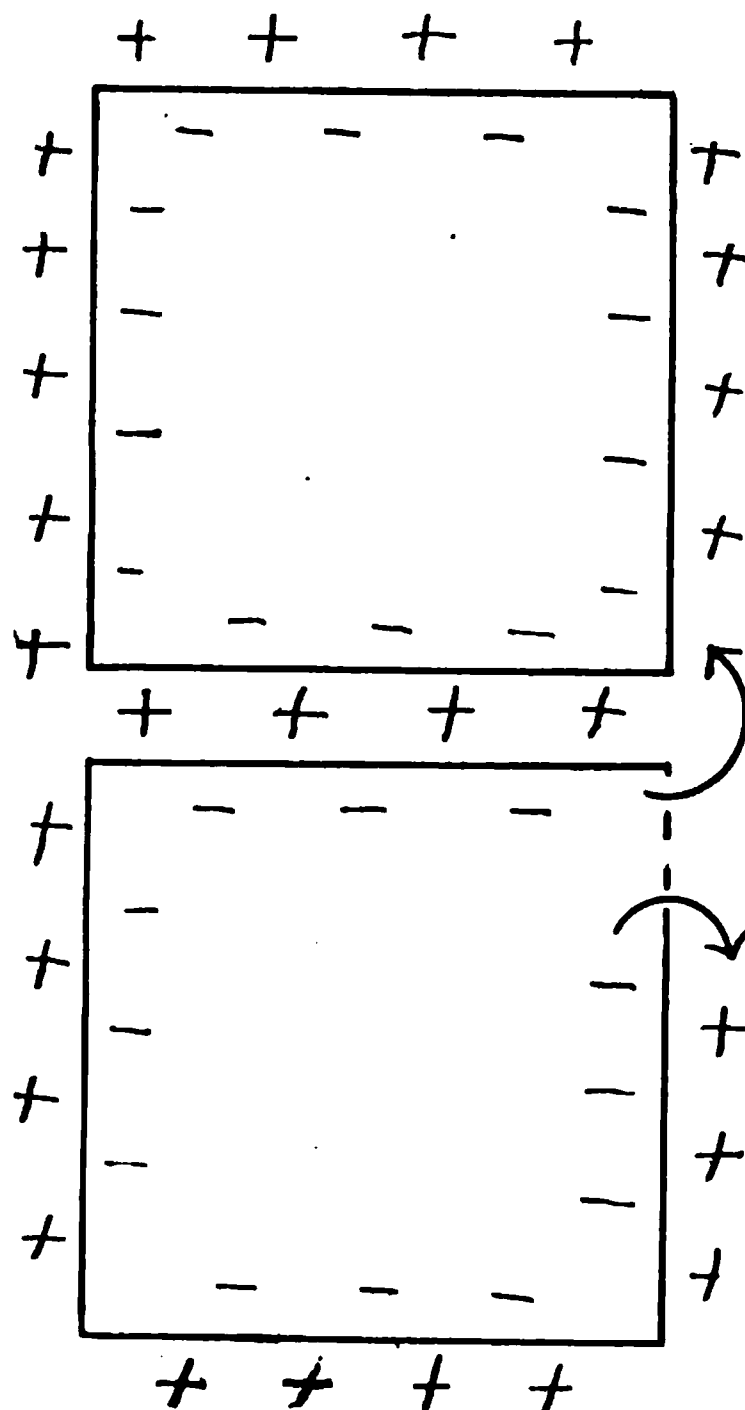


FIG. 2.

The squares represent the plasma membranes of adjacent cells. For further explanation see Fig. 1.

at one point, soon stopped the whole stream thus simulating the propagation of the stimulus.

IV. NARCOSIS.

If stimulation consists in increase in permeability, we should expect anæsthetics to prevent this change. The object of this chapter is to present evidence that may support or refute such a hypothesis.

Overton observed that warm- and cold-blooded vertebrates, insects and entomostraca, require practically the same concentration of the anæsthetic for narcosis. Certain groups of

worms require double, and protozoa and plants six times this concentration. We might conclude from this that nerves (and especially medullated nerves?) are more susceptible to narcosis than are other cells. All groups of worms contain nerves, but Loeb has shown that certain worms may perform coördinated movements after the nerves are cut, hence the higher concentration of the narcotic required to quiet them. However it should be remembered that *over-stimulation* causes rounding up and quiescence of *Amæba* and muscle may be paralyzed by increasing the permeability. The growth of plants is increased by a certain concentration of ether and retarded by a greater concentration. It may be that true narcosis (decreased permeability) of protozoa and plants cannot be produced by such substances as ether, etc.

Vertebrate nerve tissues are rich in lipoids (which have similar solubilities to neutral fats) and it is therefore significant that Overton and also Meyer¹ found that the partition coefficient of anæsthetic between olive oil and water corresponds to its anæsthetic power. Meyer² showed further, that with change of temperature, the change in the partition coefficient between oil and water, and the anæsthetic power of the substance were parallel. Pohl, Frantz, Gréhaut, and Archangelsky found that chloroform, ether, alcohol, chloral-hydrate or acetone, became more concentrated in the central nervous system than in other tissues. This is probably due to the absorption of the narcotic by the lipoids (especially the immense mass of myelin) in the nerve tissues.

If it could be proven that the plasma membrane consists of lipoids, this solubility of narcotics might be considered direct evidence for or against the permeability hypothesis, but lacking such proof we must first attack the subject from another side.

Höber³ observed that ethyl-methane, phenyl-methane, chloral-hydrate, chloroform and hypnon, in *low concentration* prevent the production by salts, of the current of injury on muscle. He showed that in *lethal doses* on the contrary these narcotics do

¹ *Arch. exp. Path. u. Pharm.*, 1889, XLII., 109.

² *Arch. exp. Path. u. Pharm.*, 1901, XLVI., 338.

³ *Pflüger's Arch.*, 1907, CXX., 492, 501, 508. Cf. R. Lillie, *Am. Jour. Physiol.*, 1912, XXIX., 373.

not prevent but even *produce* a current of injury, in this way explaining data which might otherwise seem to contradict the first statement. Galeotti and Cristina¹ observed that ether, ethyl-chlorid, and chloroform produce a current of injury on frog's muscle.

We may conclude, then, that anæsthetics, in the concentration producing narcosis, so change the plasma membrane as to prevent salts from making it permeable to anions. This is probably also true of nerve, since Höber found that ethyl-methane in low concentration prevented the sensitizing of nerve with K_2SO_4 .

Höber has attempted to connect these facts with the lipid solubility of narcotics. Moore and Roaf² had observed that *small quantities* of such narcotics as chloroform, alcohol, ether, or benzol, precipitated lipoids extracted from organs and suspended in water. But Höber and Gordon³ found that colloidal solutions of lecithin were not precipitated, but were made transparent by ether or chloroform in *high concentration*. Similarly, Goldschmidt and Pribram⁴ observed that lecithin suspended in NaCl solution, which is dissolved by chloral hydrate, methane, or cocaine, in high concentration, is precipitated by them in low concentration. On the other hand, Koch and McLean⁵ state that chloral, hypnon, acetone, or pure ether, do not change the size of colloidal particles of lecithin (*i. e.*, make them easier or more difficult to salt out). Calugareanu⁶ explains the mechanism of the precipitation of lipoids by anæsthetics by the increase in size of the particles due to absorption of the anæsthetic.

Thus there seems to be a parallel difference between the action of low and high concentrations of anæsthetics on muscle and nerve, and the action of the same on lipid suspensions, but this does not hold true for all cases. Moore and Roaf⁷ conclude that anæsthetics are bound, not only by lipoids, but also by proteids,

¹ *Arch. allg. Physiol.*, 1910, X., 1.

² *Proc. Roy. Soc. London*, 1904, LXXIII., 382; 1906, LXXVII., 86.

³ *Hofmeisters Beitrage*, 1904, V., 432.

⁴ *Zeit. f. exper. Path. u. Ther.*, 1909, VI., 1.

⁵ *Jour. Pharm. and Exp. Ther.*, 1910, II., 249.

⁶ *Biochem. Zeit.*, 1910, XXIX., 96.

⁷ *Loc. cit.*

and their charactersitic action on the permeability of the living cell may be due to their action on proteids. In other words, the plasma membrane may be entirely proteid.

It is well known that during narcosis little or no oxygen is absorbed by nerve tissue. Verworn and his pupils assumed that the narcotic directly suppressed oxidation. On the other hand Mansfeld¹ supposed that the narcotic dissolving in a lipoid plasma membrane made it less permeable to oxygen. It would be more in harmony with the phenomena considered in previous chapters, to suppose that the narcotic in low concentration decreased the permeability of the plasma membrane to the anions and molecules of some acid end product of oxidation, and thus stopped the combustion. An objection to this hypothesis is made by Warburg² who found that phenylurethan, which only slightly reduces oxidation in certain cells, fertilized eggs, delayed cell division enormously. With greater concentration of the narcotic, oxidation was greatly reduced.

V. OSMOTIC PROPERTIES OF THE BLOOD CORPUSCLES.

Hamburger and Bubonavik³ have concluded that the erythrocytes are permeable to K, Na, Ca and Mg. However, the opposite conclusion was reached by previous workers.

Gyrn's,⁴ Hedin,⁵ Traube⁶ and others observed that the erythrocytes are relatively impermeable to neutral salts (exc. NH₄ salts) amino acids, various sugars and hexite, slowly permeable to erythrite, more permeable to glycerine, and easily permeable to monovalent alcohols, aldehydes, ketones, esters, ether, and urea. In general, it may be said that the erythrocyte is permeable to lipoid-soluble substances or those that lower the surface tension of water. Such substances (for instance, ether) become more concentrated in the corpuscle than in the serum. Saponin becomes 120, and ammonia 880 times more concentrated in corpuscle than in serum.⁷

¹ *Pflüger's Arch.*, 1909, CXXIX., 69.

² *Zeit. physiol. Chem.*, LXVI., 305.

³ *Arch. internat. de Physiol.*, 1910, X., 1.

⁴ *Pflüger's Arch.*, 1896, LXIII., 86, and *Koninkl. Akad. von Wetensch. Amsterdam*, 1910, p. 347.

⁵ *Pflüger's Arch.*, 1897, LXVIII., 229; 1898, LXX., 525.

⁶ *Biochem. Zeit.*, 1908, X., 371.

⁷ Arrhenius, *Biochem. Zeit.*, 1908, XI., 161.

The erythrocytes are practically impermeable to ions. Stewart¹ observed that they offered a great resistance to the electric current. It is difficult to remove all of the serum from a mass of erythrocytes, but Bugarsky and Tangl, working independently of Stewart, obtained sediments of corpuscles having a conductivity of only 1/50 that of the serum. This indicates that the corpuscles are practically impermeable to both classes of ions, for if permeable to ions of one sign, they would probably not be such good insulators. The electric conductivity of the ash (made up to equal volume) of the corpuscles is about that of the serum, although the osmotic pressure of the solution of ash of the latter is greater.²

Hence an increase in electric conductivity of the corpuscles (as will be considered below) indicates increased permeability to ions. After the corpuscle becomes permeable to ions, further increase in conductivity might be due to liberation of ions from combinations with colloids in the interior. However many ions, for instance PO_4 , cannot be liberated without incineration or other rigorous treatment. Increase in conductivity of the blood by laking agents has been proven to be chiefly due to increased permeability of the corpuscles, since the conductivity of the serum never shows so great an increase on the addition of the laking agent, and is usually diminished (by the hæmoglobin) if the corpuscles are present.

The portion of the normal corpuscle presenting the greatest resistance to the electric current is the surface layer, since Höber³ observed that the conductivity of the interior of the corpuscle (determined by its dielectric value) is many times greater than that of the corpuscle as a whole. Peskind⁴ caused bubbles of nitrogen to form within the corpuscle and observed that they were retained by a superficial membrane. This may be the membrane which resists the electric current.

The chemical composition of the corpuscle is supposed to bear some relation to its permeability. Aside from the hæmoglobin, and the rather low water content (60 per cent.) the corpuscle

¹ *Science*, Jan. 22, 1897.

² Moore and Roaf, *Biochem. Jour.*, III., 155.

³ *Pflüger's Arch.*, 1910, CXXXIII., 237.

⁴ *Am. Jour. Physiol.*, VIII.

is composed of lecithin and cholesterin with a little nucleoproteid. It is probable that these lipoids are chemically different in different species of animals, since Lefmann¹ observed that the lipoids of erythrocytes of the same species are not toxic, whereas those of another species may be very toxic.

The distribution of these substances in the corpuscle has not been ascertained. Pascucci² supposed the corpuscle to be a bag of proteid impregnated with lecithin and cholesterin and filled with hæmoglobin. He found that artificial lecithin-cholesterin membranes were made more permeable to hæmoglobin by the laking agents, saponin, solanin and tetanus or cobra poison. Dantwitz and Landsteiner suppose the lecithin to be in combination with protein.

Hoppe-Seyler assumed the hæmoglobin to be in combination with lecithin in the corpuscle, and Bang³ has shown that lipoids may be fixed by hæmoglobin. It seems evident that there does not exist an aqueous solution of hæmoglobin within the corpuscle, since hæmoglobin crystals may be made to form in *Necturus* corpuscles without extraction of water. Furthermore, Traube and Goldenthal⁴ find that hæmoglobin has a hæmolytic action, and unless there exists some body within the corpuscle which antagonizes this action (as serum does) a hæmoglobin solution could not be retained by the corpuscle. Probably all of the so-called "stroma" constituents, not in combination with the hæmoglobin, form the plasma membrane of the corpuscle.

Under certain conditions, the hæmoglobin comes out of the corpuscles, and the blood is said to be laked. Laking of "fixed" corpuscles occurs only after the removal of the fixing reagent. Thus, sublimate-fixed corpuscles may be laked by substances which combine with mercury, such as potassium iodide, sodium hyposulphite or even serum proteids. The fact that they may be laked by heating in water is probably because the nucleo-histone is not fixed by sublimate. This process is prevented by hypertonic NaCl solution, presumably on account of its power to precipitate nucleo-histone (Stewart). Formaldehyde-fixed corpuscles may

¹ *Beiträge chem. Physiol. u. Path.*, XI., 255.

² *Hofmeister's Beiträge*, 1905, VI., 543, 552.

³ *Ergeb. d. Physiol.*, 1907, VI., 152.

⁴ *Biochem. Zeit.*, 1908, X., 390.

be laked by ammoniacal water, at a temperature which must be higher, the more thoroughly they have been fixed. Ammonia combines with formaldehyde.

Stewart¹ supposes that the hæmoglobin must be liberated from some compound before the blood can be laked. We cannot say that the corpuscle is always permeable to hæmoglobin from within outward. However the corpuscle probably is impermeable to it from without inward, since it does not take up hæmoglobin from a solution, and after the blood is laked the serum contains hæmoglobin in greater concentration than the "ghosts" do.

At any rate, permeability to hæmoglobin appears to be independent of permeability to salts, since Rollett² found that laking by condenser discharges may set free the hæmoglobin without the corpuscle becoming permeable to ions. Stewart³ concluded that the same is true of laking with sodium taurocholate (even after considering the depressing action of hæmoglobin on the conductivity).

Stewart⁴ and others had already shown that blood laked by minimal applications of such laking agents as freezing and thawing, heating (to 60°), foreign serum, and autolysis (spontaneous laking) cause but a slight increase in the permeability to ions, whereas the continued application of some of these agents, or especially such violent reagents as distilled water and saponin, cause a marked increase in electric conductivity. On the other hand, if saponin is added to defibrinated blood at 0°, the conductivity of the corpuscles to ions begins to increase before any hæmoglobin escapes from the corpuscles.

The liberation of the hæmoglobin by some laking agents may be due to the direct action of the reagent in breaking up the compound in which the blood pigment exists, but is probably sometimes a secondary effect, following increase in permeability to electrolytes.

It has been shown that many laking agents, lipoid solvents, saponin unsaturated fatty acids, soaps, and hæmolysins (containing lipase) are such as would alter lipoids physically or

¹ *Jour. Pharm. and Exper. Therapeutics*, 1909, I., 49.

² *Pflüger's Arch.*, 1900, LXXXII., 199.

³ *Am. Jour. Physiol.*, X.

⁴ *Jour. Physiol.*, 1899, XXIV., 211.

chemically, whereas pressure, trituration, shaking, heat, condensor discharges, freezing and thawing, water, drying and moistening, salts (including bile salts), acids and alkalis, might act also on proteids.

Since any treatment which causes great swelling¹ of the corpuscle leads to loss of hæmoglobin, it is probable that stretching or breaking of the surface film increases its permeability. But laking may occur without swelling, and even crenated corpuscles may be laked by sodium taurocholate.

Höber² observed that the relative action of ions in favoring hæmolysis is: salicylate > benzoate > I > NO₃, Br > Cl > SO₄ and K > Rb > Cs > Na, Li. Since this is the order in which they affect the aggregation state of colloids, their action is probably on the aggregation state of the colloids of the corpuscle (proteids or lipoids or their combinations).

The permeability of formaldehyde-fixed corpuscles to ions, is greatly increased by extraction of the lipoids with ether, or by treatment with substances such as saponin, which act on lipoids. Since the proteids have been thoroughly fixed, it is evident that they play no part in this process, though they may do so in the non-fixed corpuscles.

The relation of lipoids outside of the corpuscles to hæmolysis has been extensively investigated, and cannot be fully treated here. Willstätter found that cholesterin combines with one of the saponins, destroying its hæmolytic power. Iscovesco³ concludes that cholesterin combines with soap, and prevents its toxic action.

Changes in permeability of the corpuscles to ions were studied chemically before the application of the electrolytic method. Hamburger⁴ and Limbeck⁵ observed that when CO₂ is passed through blood, chlorine passes from serum into corpuscles and the alkalescence of the serum is increased. On the other hand, the distribution of sodium and potassium is not changed.⁶

¹ Roaf, *Q. J. Exper. Physiol.*, III., 75, supposes this swelling to be due to ionization and hence increased osmotic pressure of hæmoglobin.

² *Biochem. Zeit.*, 1908, XIV., 209, and *loc. cit.*

³ *Comptes Rendus, Soc. Biol.*, 1910, LXIX., 566.

⁴ *Zeit. f. Biol.*, 1891, XXVIII., 405.

⁵ *Arch. exp. Path.*, 1895, XXXV., 309.

⁶ Gürber, *Sitzungsber. physik.-med. Ges. Würzburg*, 1895.

Koeppel¹ and Höber² explain this process in the following manner: The lipid-soluble CO_2 enters the corpuscle, and by reacting with alkali albuminates in the protoplasm, gives off more anions than it does in the serum. During the presence of CO_2 , the corpuscle is permeable to anions, and the CO_3^- or HCO_3^- ions pass back into the serum, being exchanged for Cl^- ions to equalize the electrical potential. Sodium bicarbonate being more alkalescent than sodium chloride, the titratable alkalinity of the serum is increased.

This explanation is supported by the following facts: When CO_2 is passed through a suspension of erythrocytes in cane sugar solution the latter does not become alkaline. If CO_2 is passed through a mass of centrifuged erythrocytes, which are then added to physiological salt solution, the latter becomes more alkaline than the serum in Hamburger's experiment. Any sodium salt may be substituted for serum, and its anions will pass into the corpuscles.³ Also the number of ionic valences passing into the corpuscle is constant, *i. e.*, if sulphate is used only half as many ions enter the corpuscles as when chloride or nitrate is used. The process is reversed by removal of the CO_2 .

This same phenomenon has been observed in leucocytes by van der Schroeff.

There seems to be some relation between hæmolysis and agglutination of the corpuscles. Arrhenius⁴ supposed that agglutination by acids is due to the coagulation of the proteids of the envelope. However, since agglutination is followed by precipitation, it seems probable that the loss of the negative electric charge which tends to keep the corpuscle in suspension and causes it to repel every other corpuscle, is partly responsible for the phenomena.

The fact that water-laking is preceded by agglutination might be explained if we assume that increase in permeability to ions leads to loss of electric charge. The charge may be due to the charges on the colloids of the corpuscle or to semi-permeability to ions. The corpuscle is very poorly permeable to ions, but may

¹ *Pflüger's Arch.*, 1897, LXVII., 189.

² *Pflüger's Arch.*, 1904, CII., 196.

³ Hamburger and van Lier, *Engelmann's Arch.*, 1902, 492.

⁴ *Biochem. Zeit.*, 1907, VI., 358.

be slightly more permeable to some one ion than to others. If this ion were more concentrated in the plasma or in the corpuscle, the latter would become electrically charged, and a general increase in ionic permeability would lead to a reduction or loss of this charge. The loss of charge would favor their coming in contact with one another and their precipitation, but their cohesion is probably due to some other change, possibly the exit of adhesive substances, on increase in permeability.

VI. ABSORPTION AND SECRETION.

I. *Absorption through the Gut.*

If a live vertebrate intestine be filled with one portion of a physiological NaCl solution, and suspended in another portion of the same solution, fluid will pass through the wall of the gut from within outward. Cohnheim¹ found that holothurian gut behaves in the same way toward sea water, and the absorption stops if the gut is injured with chloroform or sodium fluoride.

It might be supposed that the hydrostatic pressure produced by the contraction of the musculature, is the driving force of absorption, but on the contrary, Reid² found that the wall of the rabbit's intestine behaved in the same way when used as a diaphragm.

Salt is absorbed by an intestine filled with a very hypotonic solution of it, and water may be absorbed when the solution is very hypertonic.

Blood salts enter the intestine when it is injured by an extremely hypertonic solution, or sodium fluoride, chinin or arsenic.

Grape sugar and sodium iodide may pass from without inwards through the wall of a normal holothurian intestine.

Traube³ claims that absorption is explained by his observation that the surface tension of the contents of the gut is less than that of the blood, but this does not apply to the experiments in which an identical solution was placed on each surface of the wall of the gut. Traube⁴ found that the addition of a substance

¹ *Zeit. physiol. Chem.*, 1901, XXXIII., 9.

² *Jour. Physiol.*, 1901, XXVI., 436.

³ *Pflüger's Arch.*, 1904, CV., 559. Cf. Iscovesco, *Comptes Rendus, Soc. Biol.*, 1911, LXXI., 637.

⁴ *Biochem. Zeit.*, 1910, XXIV., 323.

lowering the surface tension increased the absorption of NaCl by the gut.

Absorption is probably due to irreciprocal permeability of the wall of the gut. Hamburger showed that dead gut and even artificial membranes showed irreciprocal permeability to certain substances. These artificial membranes were of different composition on their opposite surfaces (parchment paper-chrome albumin, or parchment paper-collodion) and he assumed that the wall of the gut is composed of two osmotically different layers. In reality there may be more than two such layers, and the plasma membranes of the individual cells of the gut may show irreciprocal permeability.

Traube¹ showed that the rate of absorption of a substance by living gut is usually greater the more it lowers the surface tension of water. The order of ions is: $\text{Cl} > \text{Br} > \text{I} > \text{NO}_3 > \text{SO}_4$, HPO_4 and K , $\text{Na} > \text{Ca}$, Mg . The order of non-electrolytes, according to Katzenellenbogen² is: glycocoll < urea < acetone, mannit < erythrite < glycerine < acetamid, methylalcohol, propylalcohol, amylalcohol.

The rate of absorption through dead ox gut according to Hedin³ is: $\text{Br} > \text{NO}_3 > \text{Cl} > \text{SO}_4$ and $\text{K} > \text{Rb} > \text{Na} > \text{Li} > \text{Mg}$ and mannit < erythrite < glycerine < urethan < glycocoll < amylenhydrate < glycol < urea < propylalcohol < isobutylalcohol < methylalcohol, ethylalcohol.

The action of poisons on absorption may be due to the alteration of the plasma membranes of the individual cells. Mayerhofer and Stein⁴ state that even sugar in certain concentrations increased the permeability of the gut.

2. *Osmotic Relation of Aquatic Animals to the Medium.*

Fredericq found that the salt content of the body fluids of marine invertebrates is about the same as that of sea water. Henri and Lalou⁵ showed that the osmotic exchange between coelom fluid of sea urchins and holothurians and medium is chiefly

¹ *Pflüger's Arch.*, CXXXII.

² *Pflüger's Arch.*, CXIV., 522.

³ *Pflüger's Arch.*, 1899, LXXVIII., 205.

⁴ *Biochem. Zeit.*, 1910, XXVII., 376.

⁵ Winterstein, II. (2), 2.

water. If the sea water was diluted with $\frac{1}{4}$ vol. of isotonic cane sugar solution, the salt content of the coelom fluid is very little lowered in 4 hours, and only traces of sugar appear in it. The result is the same with isotonic urea (which easily penetrates most plasma membranes). But the salt content of the blood of elasmobranchs and teleosts is about half that of the sea.

Botazzi and his colleagues observed that the osmotic pressure of the blood of elasmobranchs is about equal to that of the medium, the salts in the blood being supplemented by organic substances, chiefly urea, of which there is 2-3 per cent.

If elasmobranchs are placed in concentrated sea water, the osmotic pressure of the blood rises, but the ratio of urea to salts remains the same. G. G. Scott found that changes in the density and osmotic pressure of the blood of elasmobranchs accompany changes in the salt content of the medium.

However, in marine teleosts as well as all fresh-water animals which have been studied in this respect, both salinity and osmotic pressure of the body fluids are very different from that of the medium.

The osmotic pressure of the blood of marine teleosts is about half that of the sea, but in fresh-water teleosts it is still less (but much greater than the fresh water). This indicates that there must be a change in the osmotic pressure of the blood as the fish ascends a river. Greene¹ observed that it took salmon 30-40 days to pass the brackish water, in which time they were acclimatized to fresh water. After being in fresh water 8-12 weeks, the osmotic pressure of the blood was reduced only 17.6 per cent. This reduction may be partly accounted for by the absorption of the osmotic substances in the blood by the sexual glands. In harmony with this view is the fact that the osmotic pressure of the blood of the female was reduced much more than that of the male. One salmon, that was very weak and probably dying, showed 32 per cent. decrease in Δ of blood. Sumner² observed that changes in weight and salt content of marine teleosts accompany, but are not proportional to changes in the medium.

¹ U. S. B. F., 1904, XXIV., 445; 1909, XXIX., 129; *Jour. Exp. Zool.*, 1910, IX.

² Bull. U. S. B. F., 1905, XXV., 53, and *Am. Jour. Physiol.*, 1907, XIX., 61.

Overton observed that if the cloaca and mouth of a frog in fresh water are closed, the frog constantly increases in weight. This can be prevented by the addition of .7 per cent. NaCl to the medium. In a hypotonic solution water is constantly absorbed by the skin and excreted by the kidneys. Fischer's¹ experiment, in which ligature of the leg of a frog caused great swelling below the ligature is probably to be explained by the fact that water was absorbed by the skin but could not reach the kidneys, since the blood circulation was stopped. In regard to Fischer's explanation, compare the results of Sidbury and Gies.² Sumner concluded that in the fish, the gills are the chief seat of osmotic exchange.

It appears, therefore, that osmosis occurs through the integument (including gills), kidneys and gut simultaneously, and since the contents of the gut and kidney tubules are not the same as the medium, we should not expect an osmotic equilibrium between the body fluids and the medium. Furthermore, all three of these membranes may show irreciprocal permeability.

Fresh-water fish and non-migratory marine fish are killed by great changes in the medium, even though it be very gradual.

Bert maintained that if fresh-water fish are placed in sea water, the gill capillaries contract and become blocked by the distorted corpuscles. In naked-skinned fishes, not only the gills are affected, but water may be lost from the tissues.

Bert and Sumner both agree that the salts in sea water cannot be replaced by any other substance, without causing the death of certain marine fishes. Mosso³ claimed that when sharks are placed in fresh water, the gill capillaries become so blocked with laked corpuscles that physiological salt solution could not be forced through them. He observed that the differences in the resistance of certain fish to changes in the salt content of the medium, corresponded to differences in the resistance of their blood cells to the hæmolytic action of such changes. Sumner,⁴ however, states that this blocking of gill capillaries does not occur in sharks or marine teleosts in fresh water.

¹ Fischer, M. H., "Œdema," J. Wiley & Sons, 1910.

² *Soc. Exper. Bio. and Medicine*, 1911, VII., 104.

³ *Biol. Centlb.*, 1890, X., 570.

⁴ *Proc. Seventh Internat. Zool. Congress*, Boston, 1907.

Sumner showed that as the fish becomes enfeebled by the abnormal medium, it becomes more permeable to salts.¹ Whether the direct action of the abnormal medium, or the blocking of the gill capillaries, produce the increase in permeability, has not been experimentally tested. However, the gills themselves would not be *asphyxiated* by blocking of their capillaries, and it seems probable that the change in permeability is due to the direct action of the medium.

We may conclude therefore that the death of the fish results from the osmotic exchange. This may be sufficient to cause death while the fish still maintains its normal semi-permeability, or death may occur only after increase in permeability, due to the direct action of the medium on the osmotic membranes.

A similar increase in permeability may explain Wo. Ostwald's observations on fresh-water *Gammarus* in pure salt solutions.² He found that the ratio of the rapidity of death to the concentration is about constant up to a certain point, above which it is much greater. This critical concentration has nothing to do with the osmotic pressure, since it is different for different salts. Perhaps at this concentration the salt made the membranes more permeable.

Schücking³ found that nicotine and strychnine made the skin of *Aplysia* more permeable to salts. Since cocain retarded shrinkage in hypertonic solution, he supposed that the hydrostatic pressure produced by the muscles aided shrinkage. However the hydrostatic pressure is probably very small, and the effect might have been due chiefly to an increase in permeability to salts, produced by the cocain.

3. *Secretion of Lymph and Tissue Juice.*

Höber supposes the raising of the osmotic pressure by the katabolism of the tissues, causes fluid to be drawn out of the blood-vessels, and states that the lymph in the thoracic duct has a greater osmotic pressure than the blood.

Traube states that the surface tension of transudates and

¹ Cf. Greene, above.

² *Pflüger's Arch.*, 1905, CVI., 568.

³ *Arch. Anat. Physiol.*, Physiol. Abt., 1902, 533.

exudates is always greater than that of the blood. He cites a case in which a transudate was caused to be absorbed by injecting into it a substance which decreased its surface tension.

4. *Excretion.*

Milk and bile have about the same osmotic pressure as the blood, but urine is almost dry in some animals; it is usually hypertonic in man but may be hypotonic.

Traube maintains that the surface tension of the normal urine is always greater than that of the blood, and that this is the driving force in excretion.

However, Höber and others suppose that the substances to be excreted may be formed into solid bodies in the tubule cells, and thrown out into the lumen.

If lipoid-insoluble dyes are fed to frogs, granules in the cells of certain segments of the kidney tubule are stained with them. The dye is not first excreted by the glomeruli and then absorbed from the lumen by the tubule cells, for if the vena Jacobsoni, which supplies the tubules, is ligatured, no staining occurs, although the renal arteries still supply the glomeruli.

The stained granules in the tubule cells are thrown out into the lumen and pass into the bladder. These granules usually dissolve to form a slimy substance in the urine, but some of them may remain intact.

The circulation in mammalian kidneys cannot be controlled in the same way, but after intravenous injection of a certain lipoid-insoluble dye, no stain may be detected in the walls of the glomeruli, although the tubule cells are stained. The stain in the lumen does not appear above the level of the stained tubule cells. In the excretion of carmine, it may be found in granules in the tubule cells and lumen, similar to those found in frog's kidneys.

It has been supposed that urea is excreted by collecting in these granules and passing out with them, but it would be even simpler to assume that some substance is excreted into the lumen, which combines with urea and so lowers the concentration of that in solution, thus accelerating its excretion.

The chief recommendation for the granules is their valve-like

action, which would account for the secretion of urine against a concentration gradient, but a simpler mechanism of such a process is shown in Hamburger's double membranes.

The blood pressure may aid in the secretion of the water of the urine, which is eliminated chiefly through the glomeruli, but its insignificance in the elimination of urea is shown by the fact that after increasing the volume (and therefore pressure) of rabbit's blood 70 per cent. by transfusion, the urea elimination was not or only very slightly increased.

VII. CELL DIVISION.

Various hypotheses as to the cause of cell division have been advanced by the morphologists. Hertwig, supposed that when the ratio of nucleus to cytoplasm is less than normal, the cell will divide.¹ Gerassimow² subjected cells of *Spirogyra* to low temperatures and other abnormal conditions and obtained an increased amount of chromatin in some of them. These cells did not divide until the ratio of nucleus to cytoplasm was as great as at the time of division of a normal cell.

I found that chromatin is not necessary for cell division.³ After extracting the chromosomes from the starfish egg, I caused it to divide. In this case the ratio of nucleus to cytoplasm was zero; however the cell did not continue to divide indefinitely.

There is no easy method of determining the ratio of nucleus to cytoplasm. Some cells contain large vacuoles whose contents are not considered as cytoplasm. Eggs contain fat drops and granules compounded of protein and lipoids. These are not considered as cytoplasm by all investigators. If the granules and oil are included as cytoplasm, the ratio of nucleus to cytoplasm is very small, and yet the egg cell does not divide unless "stimulated" by the sperm or some other means.

R. Lillie⁴ observed that chemical substances, which in low concentration cause the *Arbacia* egg to divide, in high concentration cause outward diffusion of the red pigment (echinochrome) and compared this to the laking of erythrocytes.

¹ He is not confirmed by Conklin, *Jour. Exper. Zool.*, 1912, XII., 1.

² *Bull. Soc. Imp. Nat.*, Moskau, 1904, No. 1.

³ McClendon, *Arch. f. Entwicklungsmech.*, 1908, XXVI, 662.

⁴ *BIOL. BULL.*, 1909, XVII., 188.

This is made more striking by the fact, mentioned first by Loeb, that hæmolytic agents are effective in artificial parthenogenesis. R. Lillie observed that pure solutions of sodium salts caused the egg to divide, the order of effectiveness of anions being $\text{Cl} < \text{Br} < \text{ClO}_3 < \text{NO}_3 < \text{CNS} < \text{I}$. He also found that these salts could be inhibited by others (CaCl_2 , MgCl_2), as is characteristic of the antagonistic effects of salts in physiological phenomena, and the precipitation of colloids.

I found that the sea urchin's egg contains fatty substances, and relatively large amounts of lecithin probably in combination with proteids. I found that *Toxopneustes* eggs freed from the jelly-like coverings, contained about 10 per cent. lecithin (alcohol extract ppt. with acetone) and about 2 per cent. of an extract soluble in alcohol or acetone and containing rosettes of fat-like crystals. This extract blackened strongly with osmic tetroxide and effervesced on adding dry Na-carbonate in water, then emulsified, probably it contained unsaturated fatty acid.

According to a private communication by Mathews, the egg of the starfish contains lecithin and an unsaturated fatty acid, but no cholesterin. In this last characteristic it differs markedly from the erythrocyte. There is no way of determining whether these substances enter into the composition of the plasma membrane, but the facts are presented in order to indicate the possibilities.

We have seen that the exit of hæmoglobin is probably not due to increased permeability to this substance. It is possible that the same is true of echinochrome. I found that the echinochrome in the egg shows a continuous spectrum, whereas that extracted in various ways shows characteristic bands. It may possibly be held by chemical combination in the egg.

However I found other evidence for increase in permeability of the sea urchin's egg coincident with beginning development:¹

1. Fertilized eggs are caused to shrink more quickly than unfertilized eggs, with isotonic sugar solution. Presumably the fertilized eggs are more permeable to the substances exerting the internal osmotic pressure.

2. The electric conductivity of the egg increases about $\frac{1}{4}$ when

¹ McClendon, *Amer. Jour. Physiol.*, 1910, XXVII., 240.

it is fertilized or made parthenogenetic with acetic acid, indicating increased permeability to ions.

Lyon and Shackell¹ and Harvey² observed that methylene blue and neutral red enter fertilized eggs more quickly than unfertilized eggs. Harvey supposed that only the free color base (undissociated) entered, since the addition of a little acid to the sea water prevented the staining of the eggs.

Mathews³ considered the penetration of stains into the egg as a chemical process (the stain forming a salt combination with the lecithin or proteins of the egg surface).

Harvey observed, further, that NaOH penetrates fertilized more easily than unfertilized eggs, but the eggs are killed by the alkali.

The fact that the unfertilized frog's egg continues to swell *for a long time* in water (Biataszewitz) whereas the osmotic pressure of the fertilized frog's egg is *quickly* reduced to equal that of the medium (Backmann and Runnström) indicates increase in permeability to osmotic substances on fertilization. In this connection it is interesting to note that Bataillon,⁴ Brachet, and myself⁵ caused the unfertilized frog's egg to rotate normally and segment merely by pricking it.

It has been supposed by various observers that the "formation" of the fertilization membrane is very closely related to the segmentation of the egg. Loeb observed that the sea urchin's egg may develop without the formation of a fertilization membrane, and I have confirmed this observation, and shown that it is very probably wrong to suppose that this is a case of failure in "pushing out" of the membrane. Apparently "membrane formation" is not essential for the segmentation of the egg, although by furnishing protection it may insure the development of the embryo.

Loeb postulated that an osmotically active colloid exists in the unfertilized egg, but is so covered with lipoids that it does not absorb water until it is squeezed out or otherwise exposed

¹ *Science*, 1910, XXXII., 250.

² *Ibid.*, p. 565.

³ *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

⁴ *Arch. Zool. Expér.*, 1910 (5), VI., 101.

⁵ McClendon, *Amer. Jour. Physiol.*, 1912, XXIX., 298.

at the surface of the egg, at the beginning of development (when it fills the so-called "perivitelline space"). I observed that this substance bears a positive charge (is basic) since it migrates toward the kathode when an electric current is passed through sea water containing the fertilized egg.

The unfertilized egg is imbedded in a mass of jelly which is probably mucin. This jelly bears a negative charge (is acid) since it combines with color bases.

When the positively charged colloid is exposed at the surface (on increase in permeability) and comes in contact with the negatively charged jelly, the two mutually precipitate at their surface of contact, thus forming the fertilization membrane. But if all of the jelly is washed off of the egg before the latter is caused to develop, no fertilization membrane is formed (as I have observed) because no two oppositely charged colloids are brought in contact, but the basic colloid may with difficulty be seen as a refractive layer, which has been mistaken for a poorly developed "fertilization membrane."

The observation of Lyon¹ makes it appear that catalase comes out of fertilized more quickly than unfertilized eggs, probably due to increased permeability.

Lyon observed that CO₂ came out of fertilized more quickly than unfertilized eggs, and O. Warburg, Loeb and myself² observed that oxygen is absorbed more rapidly by the former. We might ask: Does increased permeability allow increased oxidation, or is increased oxidation the primary cause of the increased respiration?

The permeability change is the simplest explanation, and in what other way could oxidation be increased? Loeb supposed the sperm carried an oxidase into the egg.³ But no addition of oxidase is concerned in artificial parthenogenesis, and Loeb assumed that the oxidase (or other enzyme, kinase?) is held in the egg periphery and cannot penetrate the egg interior until the permeability is increased.

In addition to oxygen, oxidase, and escape of CO₂, hydroxyl

¹ *Am. Jour. Physiol.*, 1909, IV., 199.

² McClendon and Mitchell, *Jour. Biol. Chem.*, 1912, X., 459.

³ In this connection it is interesting to note that Masing, *Zeit. physiol. Chem.*, 1910, LXVI., 265, failed to find more iron in sperm than in sea water.

ions are necessary for the rapid oxidation of the sea urchin egg (Loeb), and Harvey showed that the unfertilized egg is practically impermeable to OH ions of low concentration. The increased permeability allows hydroxyl ions in the sea water to penetrate the egg, as shown by Harvey, and, since the sea is always alkaline, this may explain the increased oxidation.

Asters always develop in the egg before segmentation. In the normal egg these have some relation to the division of the nucleus, but even if a nucleus is not present, I have observed that the cytoplasm constricts along a line on the surface farthest removed from the centers of the asters.

The constriction of the cytoplasm is probably due to a band of increased surface tension (or to decreased surface tension at the poles). This might be caused by local increase in permeability to ions, causing decreased polarization, at the equator (or increased polarization at the poles, due to increased production of the polarizing electrolyte in the asters).

The same reasons that were given for assuming that the surface of the *Amæba* is electrically polarized, hold good for the egg. The first change is probably a general increase in surface tension, indicated by rounding up of the egg. Later this may become localized from internal causes and result in cleavage.

Hyde¹ observed local changes in electric polarization of *Fundulus* eggs during cleavage, indicating that surface tension changes and cleavage are due to this cause.

It has been objected that the segmentation of the egg is not a typical case of cell division, since the egg cell is "wound up" and ready for some "stimulus" to set it going, whereas tissue cells must "grow" or "rest" after each division before dividing again.

It may be true that growth is prerequisite to division, but this cannot be formulated quantitatively. In the spore-formation of certain organisms, a cell may divide in a relatively short time into myriads of almost ultra-microscopic cells.

Hertwig may be right, in general, in assuming that the relative growth of nucleus and cytoplasm influences division, but the difficulties in proving this have been indicated, and this cannot

¹ *Am. Jour. Physiol.*, XII., 241.

be expressed in chemical terms. It is generally supposed that nucleic acid is a more abundant constituent of the nucleus than of the cytoplasm, but much evidence has appeared for believing that it is often present in considerable quantities in the cytoplasm. Loeb supposed that the segmentation of the sea urchin egg is accompanied by an "autocatalytic" synthesis of nucleic acid, since the nuclei increased in number. But Masing¹ and more recently Shackell² by chemical analysis found as much nucleic acid in the unsegmented egg or 1-cell stage as in the blastula stage.

There is some indirect evidence that increase in permeability may cause an increased division rate of tissue cells. Though cell growth may influence division, it is probable that permeability influences growth.

Various "stimuli" cause increased proliferation of cells of the germinal layer of the skin. It is commonly known that mechanical stimuli increase growth of the skin.

Bernhard Fisher observed that Sudan III. or Scharlack R³ cause increased proliferation of the epidermis. When the dye is injected under the skin of a rabbit the skin grows toward the dye.

Furst⁴ found that gradual increase of temperature caused a corresponding increase in proliferation of tissue cells (due to increased chemical reaction and inflammation of the tissue). But when a certain temperature was reached a sudden jump in the increase in proliferation was observed without a corresponding increase in inflammation. This is similar to the phenomenon seen in unfertilized eggs, where a rise in temperature beyond a certain point causes segmentation.

It has also been observed that electrical stimulation may cause increased proliferation of tissue cells.

All of these changes (electrical, thermal, or mechanical stimulation, or treatment with lipoid soluble substances) cause in-

¹ *Zeit. physiol. Chem.*, 1910, LXXVII., 161.

² *Science*, 1911, n. s., XXXIV., 573.

³ Which are practically insoluble in water but soluble in fats and lipoids and, as I have observed, slightly in lipoid-protein combinations.

⁴ See v. Dungern u. Werner, "Das Wesen Bösartigen Geschwülste," Leipzig, 1907, p. 65.

creased permeability and segmentation of the sea urchin's egg. Therefore, from analogy, we may conclude that increase in permeability may cause tissue cells to divide.

The "wound stimulus" to regeneration of tissue may also cause increased permeability of the cells.

In a preceding chapter it was shown that the "current of injury" produced by the negative electric potential of a wounded surface is common to animal and plant tissues. The wounded cell acts as an electric generator and a current flows through neighboring cells.

I observed that if a current is passed through living tissue, which is subsequently fixed and stained, basophile substances will be found displaced toward the anode. In sections of tissue adjacent to a wound the extent of the current is indicated by the displacement of basophile granules. The current affects first the cells in contact with the wounded cells, then extends in some directions more than others. Electric currents ("currents of growth") continue for many days after the wound has healed.

Since electric currents cause sea-urchin eggs and tissue cells to divide and proliferate, probably these bio-electric currents constitute the so-called "formative stimulus" of regeneration.

Embryonic cells, cells of germinal regions, and cancer cells are distinguished by their great power of proliferation, or rapid division. It is probable that the plasma membranes of these cells are more permeable than those of other tissue cells in the same medium or under the same conditions.

Cancers have been produced by the action of X-rays (electric pulsations) on the skin. The cells in the skin were so changed that they proliferated more rapidly. Similarly, electric changes have been observed to start the egg cell to rapid proliferation. There is probably some irreversible change in the permeability of these cells, which does not, however, make the plasma membrane incapable of subsequent reversible changes in permeability (*i. e.*, the change is unlike what occurs at death of the cell).

The suggestion that cancer cells are more permeable than tissue cells in general may possibly be of therapeutic importance. Loeb has shown that fertilized eggs are more sensitive than unfertilized eggs to various toxic substances (probably because

these substances enter the fertilized eggs more easily). The same explanation may possibly be applied to the effect of sugar on certain living cells. The unfertilized eggs of the frog, *petromyzon*, sea urchin and annelid have been caused to segment, by placing them in sugar solutions. Mayerhofer and Stein¹ observed that sugar in certain concentrations increased the permeability of the gut to certain salts, and in this condition the gut was more easily injured by the diffusion of substances.

Similarly Stockard observed that sugar increased the toxicity of pure solutions of salts on the *Fundulus* egg. Morgan and Stockard² showed that this was not due to the inversion of sugar or to the osmotic pressure, and supposed that the sugar might combine chemically with the salt. It seems probable that the sugar increased the permeability to salt. The fact that sugar in fresh water is toxic whereas the same amount of sugar in the normal medium (sea water) is not toxic or less toxic, indicates that the salts within the *Fundulus* egg are the same as those outside (in sea water), and increase in permeability to them does not lead to diffusion while they remain in sea water, but diffusion takes place in fresh water.³

If it be shown that cancer cells are more permeable, substances may be found which kill cancer cells more easily than tissue cells as explained below.

Whereas a certain increase in permeability of the cell seems to cause division, a very great increase in permeability causes death (hæmolysis, cytolysis, bacteriolysis). It has been shown that certain lysins are specific for certain cells, probably because the plasma membranes of these cells differ chemically.

The fertilized egg is more easily cytolized than the unfertilized egg by certain substances. It therefore appears that the more permeable the cell is in the beginning, the more easily is the permeability brought to the point which causes cytolysis.

Hence it is probable that certain substances may be found by which cancer cells can be more easily cytolized than normal tissue cells.

¹ *Biochem. Zeit.*, 1910, XXVII., 376.

² *BIOL. BULL.*, 1907, XIII., 272.


³ In the absence of sugar I have shown that no diffusion takes place in fresh water. *Amer. Jour. Physiol.*, 1912, XXIX., 295.

It has been shown that narcosis is accompanied by decreased permeability. On the other hand, certain forms of inhibition of muscle are accompanied by an increase in permeability. May certain cells be inhibited in proliferation by an increase in permeability, too great for cell division but not great enough for cytolysis? The great oxidation rate in eggs inhibited in cleavage by very hypertonic solutions as determined by Warburg, seem to indicate this.

It has been shown that certain tissue cells inhibit the proliferation of others. In the healing of wounds, the epidermis inhibits the growth of connective tissue. If a wound remains uncovered by epidermis for a relatively long time, processes of connective tissue may grow outward, but this is prevented by the growth or transplantation of epidermis over the wound.

Perhaps the proliferation of the connective tissue is due to abnormal "stimuli" (bio-electric currents, diffusion of substances) such as cause proliferation in regenerating tissue generally. The presence of epidermis over the wound might protect the connective tissue from these "stimuli."

The foregoing facts and the speculations based on them may not be of far-reaching importance in themselves, but they suggest lines of research, which if followed, it is hoped, will add a great deal to cell physiology and pathology and be an aid to the understanding of many problems in therapeutics.



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Laws of Surface Tension and their Applicability to Living Cells and Cell Division

by

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With 10 figures in text

Sonderabdruck aus dem

Archiv für Entwicklungsmechanik der Organismen

Herausgegeben von Prof. Dr. Dr. Wilh. Roux in Halle a/S.

XXXVII. Band, 2. Heft

Ausgegeben am 26. August 1913

Leipzig und Berlin

Wilhelm Engelmann

1913

Das Archiv für Entwicklungsmechanik der Organismen

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Der Herausgeber:

Prof. Dr. Wilh. Roux,

HALLE a/S. (Deutschland)

Der Verleger:

Wilhelm Engelmann,

LEIPZIG UND BERLIN.

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The Laws of Surface Tension and their Applicability to Living Cells and Cell Division.

By

J. F. McClendon.

Contribution from the Embryological Laboratory of the Cornell
University Medical College.

With 10 figures in text.

Eingegangen am 21. April 1913.

QUINCKE long ago interpreted certain movements of drops of fluid as due to surface tension changes and suggested that this might also apply to living cells. BÜTSCHLI extended this application to cell division. However, since Biologists are not all of the same opinion on this subject, I have ventured to review it from the standpoint of dynamics, based on the molecular hypothesis. Some of the principles for which dynamical proofs are given in this paper were first established by thermodynamical proofs. However, it seems simpler to omit thermodynamics wherever unnecessary. This paper claims no originality, but references to original sources are omitted for the sake of brevity, the author taking in such cases the whole responsibility for the correctness of the deductions. Since T. B. ROBERTSON has opposite views from those of QUINCKE and BÜTSCHLI, it seems desirable to consider especially ROBERTSON's latest paper¹⁾ and all page numbers refer to his article.

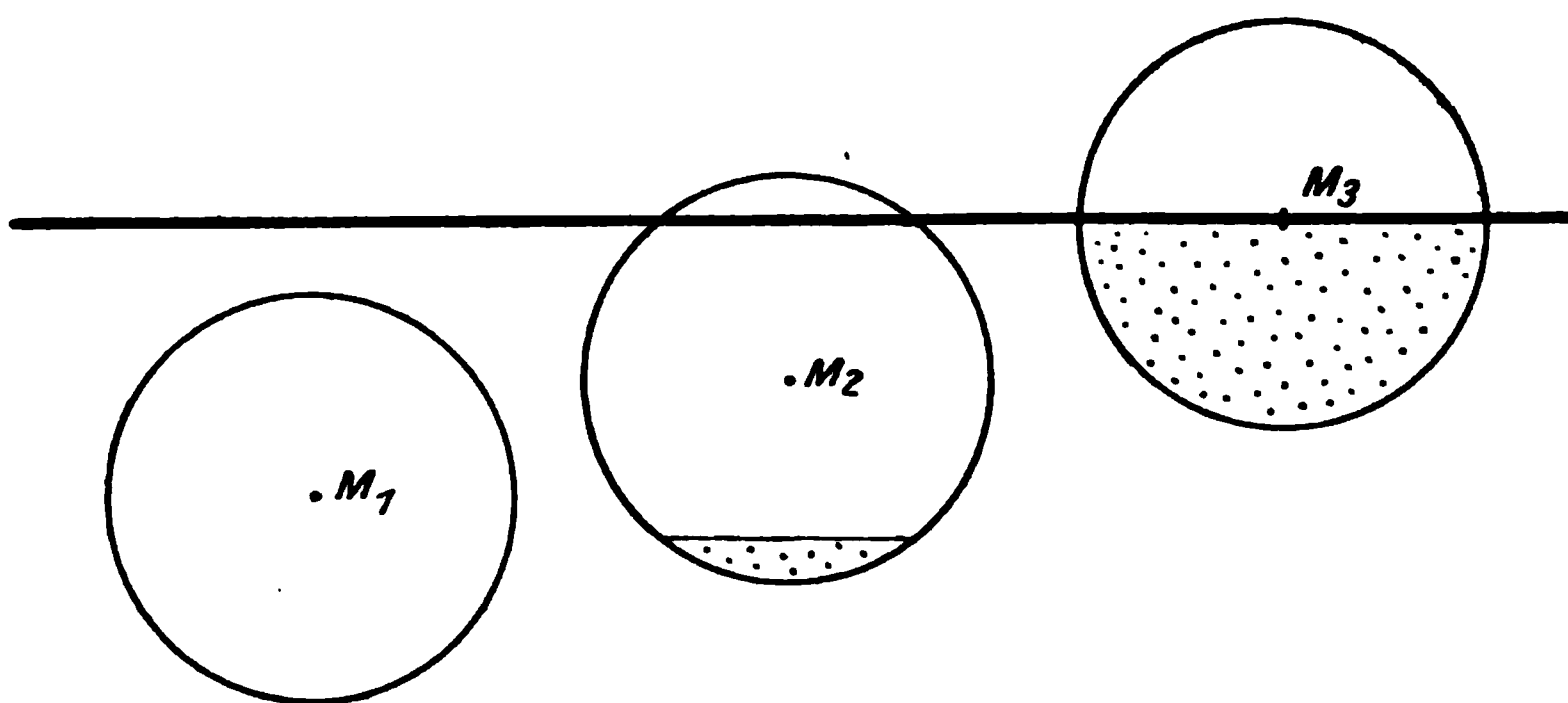
Within a liquid, the molecules are attracted by one another, but the force of this attraction decreases rapidly with the distance. According to QUINCKE, molecular attraction is not appreciable at a distance greater than $.05 \mu$. If a sphere be described about a mole-

¹⁾ Arch. f. Entw.-Mech. Bd. 35. 1913. S. 692.

cule, with a radius equal to the range of molecular attraction, we may call it the sphere of molecular attraction.

In Fig. 1 the upper surface of a liquid is represented by the horizontal line and 3 molecules, M_1 , M_2 , M_3 , by dots around each of which is a circle representing the sphere of molecular attraction. Since the attraction of molecules outside this circle is imperceptible, we may consider each molecule as attracted only by all other molecules in its circle. The molecular attractions on M_1 are equal on all sides and consequently neutralize one another. The upper part of the circle around M_2 is out of the liquid and an equal and opposite part of the circle (the stippled area) contains molecules whose downward attraction on M_2 is not compensated by an upward at-

Fig. 1.



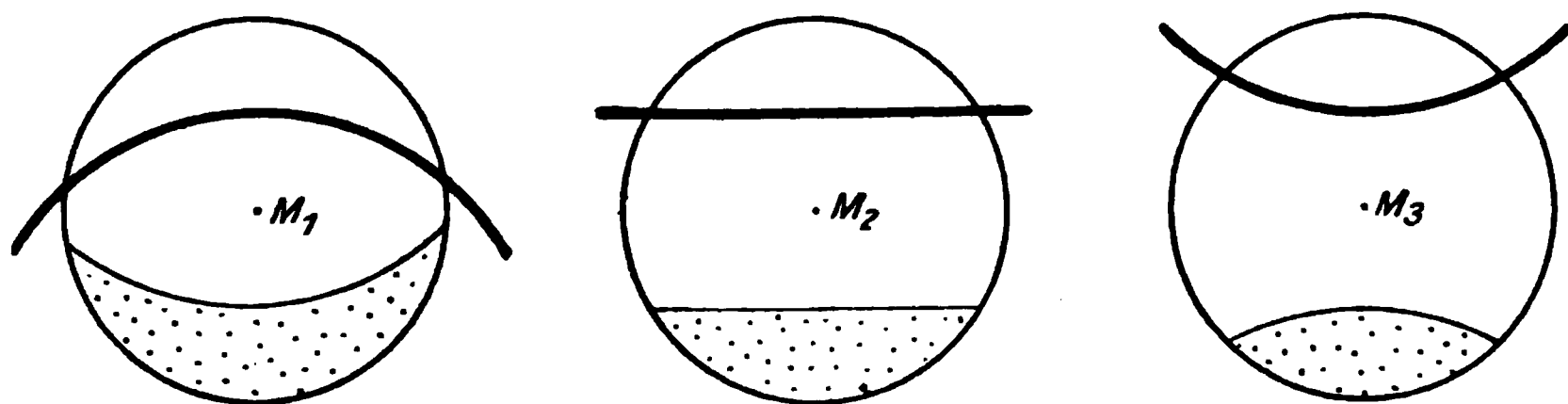
traction. The molecule M_3 is attracted downward only. This downward attraction on all molecules in a surface film equal in thickness to the range of molecular attraction pulls them closer together. The closer they are pulled together the greater their attraction for one another. Hence, the surface film of molecules under tension acts like a stretched skin or membrane.

The magnitude of this surface tension depends on the magnitude of molecular attraction, which in turn depends on the chemical composition of the liquid, and also upon the temperature and pressure, since these affect the distance between the molecules. Surface tension also depends on the form of the liquid surface, as may be deduced from the following:

In Fig. 2 let M_1 , M_2 , M_3 represent three molecules each of which is the same distance beneath a liquid surface, represented by the heavy line above, which is plane over M_2 , convex over M_1 and con-

cave over M_3 . Around each molecule the sphere of molecular attraction is represented by a circle. Very much of the volume of the sphere of M_1 projects above the surface, whereas less of the volume of M_2 and least of the volume of M_3 project. With the same reasoning as applied to Fig. 1 we may conclude that the downward attraction is greatest on M_1 , less on M_2 and least on M_3 . Hence surface tension is greatest on a convex surface, less on a plane surface and least on a concave surface. It should be remembered that every curved surface cannot be classed as convex or concave. The curved surface of a sphere or cylinder is convex when viewed from without and concave when viewed from within. However, the constricted portion of an hour glass when viewed from without appears concave along a meridian and convex along the equator. When the meridional concaveness is as great as the equatorial convexness of the sur-

Fig. 2.



face film of such a (fluid) body, no inward or outward force is exerted, as the result of surface tension. We have then what might be called a >surface of no curviture<.

If the liquid under consideration is in absolute vacuum the foregoing scheme is complete, but if molecules of any kind lie above the surface of the liquid they must be taken under consideration. If the liquid is covered by a second liquid, which for purposes of distinction we will call the medium, and the attraction of the molecules of the medium for those of the liquid is less than the attraction of the molecules of the liquid for one another, the downward tension on the molecules M_2 and M_3 in Fig. 1 and M_1 , M_2 and M_3 in Fig. 2 will be decreased by the upward pull of the molecules of the medium filling the part of the sphere of molecular attraction which lies above the surface of the liquid. Hence the surface tension in all cases will be decreased.

For purposes of description one of the two liquids in contact has been called the medium, but it should be remembered that both liquids display surface tension, and what is usually called the sur-

face tension of a drop of one fluid submerged in another, is really the sum of the tensions of both fluids at their surface of contact. Therefore in considering the effect of curvature of a surface on its tension we must know which of the two liquids has the highest tension at their surface of contact, a fact which is ignored by ROBERTSON (page 699). Where there is not an appreciable difference between these two tensions, the effect of curvature is negligible, since it would cause reciprocal changes in the two tensions. Therefore the best method of determining the effect of curvature in any given case is direct experiment, and this gives a method for determining which of the two liquids has the greatest surface tension.

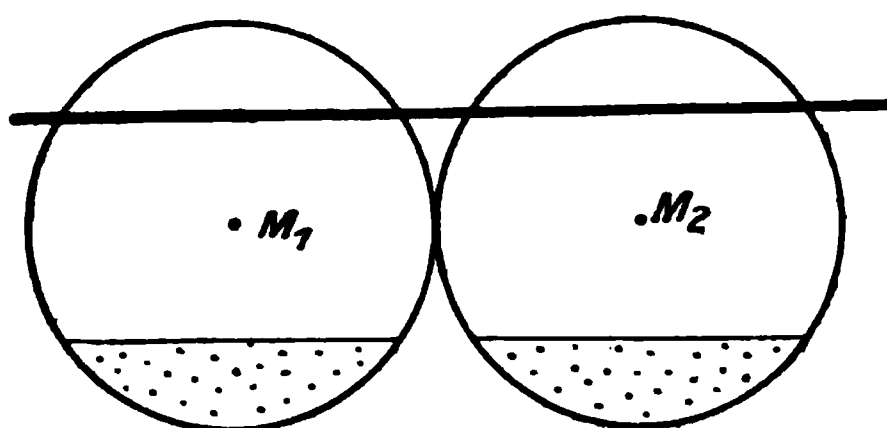
If there is dissolved in the liquid a substance whose molecules are attracted by the medium more strongly than the molecules of the solvent are attracted, the molecules of the solute that lie in the surface film will increase the upward pull of the medium and hence decrease the surface tension of the liquid (and of the medium). Also the solute will become more concentrated in the surface film than in the interior of the liquid. If the medium attracts the molecules of the solute less than the molecules of the solvent, these latter will tend to displace the molecules of the solute from the surface film. But those molecules of the solute, which by their kinetic energy force their way into the surface film, will raise the surface tension. Therefore, the raising of surface tension by dissolved substances requires the introduction into the surface film of (kinetic) energy from without. This is a fact which is entirely neglected by ROBERTSON on page 696 in which he says in criticism of my work "without the introduction of energy from without, a phenomenon which is thermodynamically impossible".

In so far as molecular attraction determines solubility (and we will assume that it is one factor in solubility) we may conclude that, if the molecules of the liquid are attracted by those of the medium as strongly as or more strongly than by one another, the liquid will mix with the medium and there will be no surface for consideration. Also if the molecules of a solute are attracted more strongly by those of the medium than by those of the solvent, the solute will diffuse into the medium. (Our proof of this is based on the assumption that molecular attraction is a factor in solubility. It is important to note that WILLARD GIBBS has furnished thermodynamical proofs of essentially the same conceptions and they have also been substantiated by considerable experimental evidence.)

A gaseous medium behaves in the same way as a liquid medium save for the fact that its molecules are so widely dispersed that its effect upon a liquid surface film is less marked. Hence the surface of a dense and poorly volatile liquid in contact with air always has a high tension.

In case of a solid ›medium‹ the surface film loses its similarity to a stretched skin by being attached to the solid. Also, there is perhaps another peculiarity. Often liquids do not dissolve or are not absorbed by solids, even though the molecules of the liquid are attracted by those of the solid more strongly than by each other (adhesion). In the latter case, the molecules in the surface film of the liquid would be attracted more strongly toward the solid than toward the remainder of the liquid and hence be brought closer together and form a surface tension film. Also, if molecules of a solute are attracted by this solid more strongly than by the molecules of the liquid, they would become more concentrated in the surface film (adsorbed) and yet increase the surface tension.

Fig. 3.

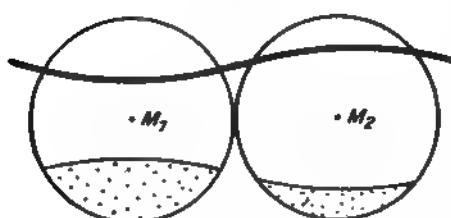


In any continuous surface of uniform curvature, if the surface tension is not uniform, surface movements will result. If the tension is increased over any area, that area will ›contract‹; consequently, if the tension is decreased over any area the pull of the remaining surface will cause the area of less tension to spread or ›expand‹.

This self evident fact may be analysed as follows: let the heavy horizontal line in Fig. 3 represent the upper surface of a liquid, and M_1 and M_2 two molecules at equal distances below the surface, and the circles about them the spheres of molecular attraction. Both M_1 and M_2 are attracted downward by a force equal to the attraction of the molecules in the stippled area of its sphere. However, suppose by a local decrease in temperature the molecules around M_2 are brought closer together and hence the number of molecules in the sphere of M_2 increased (causing increase of surface tension), then molecules from the adjacent regions are attracted toward the sphere of M_2 and the surface above M_2 is raised and made convex, while the surface above M_1 is lowered and made concave, as in Fig. 4.

Now M_2 is pulled downward by the molecules of a smaller (stippled) region than M_1 , but they are more crowded. Suppose the downward attractions on M_1 and M_2 are exactly the same. One might suppose that the difference in surface tension is also equalized, and ROBERTSON, on page 699, falls into this error (see his Fig. 2). However, M_1 is now nearer the surface than M_2 and a new figure must be drawn in which they are equidistant from the surface (Fig. 5). It may

Fig. 4.

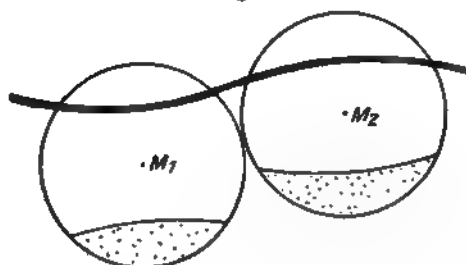


readily be seen by inspecting Fig. 5 that the surface tension is not equalized but that the difference is increased, and the surface tension cannot be equalized until the molecular attraction per unit volume is equalized. In other words surface movements will con-

tinue until the chemical composition and temperature of the surface are uniform. (Whereas Figs. 3 and 5 serve to analyse surface movements, they do not apply to changes in curvature resulting from such movements, since the internal pressure of the fluid is left out of consideration.)

These surface movements, by friction on the adjacent fluids, cause more general movements. In case of a drop suspended in a

Fig. 5.



liquid medium, spreading of the surface of one polar area will cause a vortex movement in the drop and another in the medium. Because of the inertia of the medium, these movements would lead to locomotion of the drop. (ROBERTSON neglects this factor in his

erroneous consideration of locomotion of the *Amoeba*, page 705.)

Since the surface film acts as a stretched skin it exerts no force perpendicular to the surface if that is plane. However, if the surface is concave, an upward pull is exerted and if it is convex a downward (inward) pressure is produced on the liquid beneath. The pressure within a spherical drop may be calculated as follows: Let R equal the radius and P the internal pressure produced by the

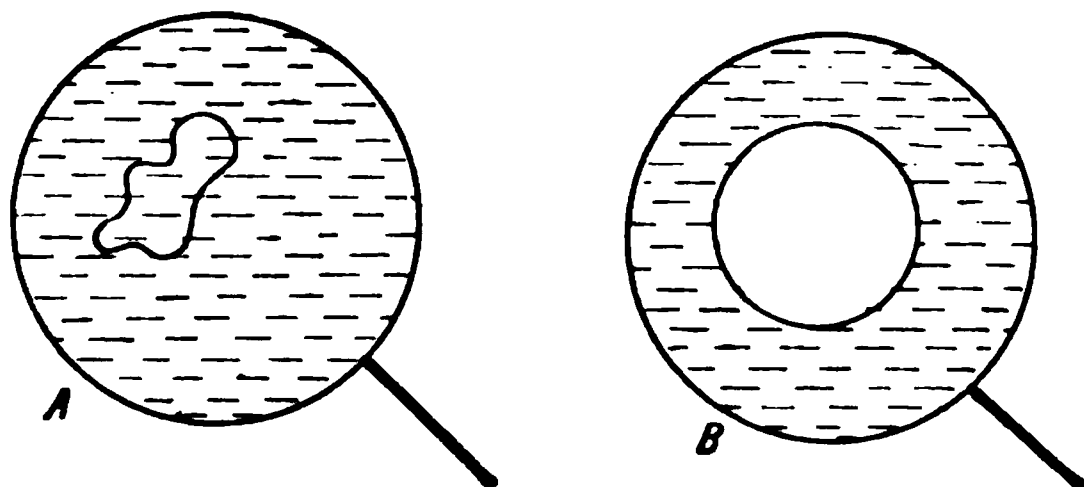
inward pressure of the surface film. If the sphere is divided into two hemispheres by a rigid plane partition, the area of this partition will be πR^2 and the downward force on the upper surface of the partition, $\pi R^2 P$. The film meets the partition at right angles along the circumference of a 'great circle', whose length $= 2 \pi R$. Hence if T is the surface tension of the liquid, the upward force exerted by the surface tension on the partition is $2 \pi RT$. Hence, since this upward force must be equal to the downward pressure, we have:

$$\pi R^2 P = 2 \pi RT$$

$$P = \frac{2 T}{R}.$$

Surface tension may be illustrated by the following experiment. A wire ring is dipped into soap solution and withdrawn, so that the

Fig. 6.



hole in the ring is closed by a liquid film. On this film a loop of thread is laid (Fig. 6, A). The film is then punctured within the loop and the film outside the thread pulling in all directions toward the wire ring, stretches the loop into a circular form (B).

The spherical form of drops of a liquid is due to the tension of the surface film which acts as a stretched skin.

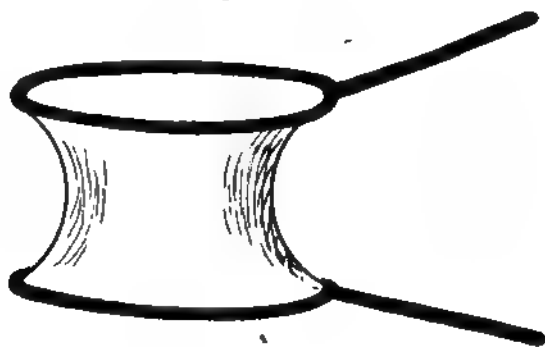
Surface tension of a liquid may be measured in the following manner: The liquid is sucked up into a pipette, the other circumference of whose mouth is measured in centimeters. The fluid is allowed to flow out slowly until a drop falls off. The drop is weighed in grams. The weight divided by the circumference is the surface tension, since a surface film, the length of whose cross section equals the circumference, supports that weight. If the measurement is made at the Earth's equator the above quotient may be reduced to dynes by multiplying by 978.

A 'surface film of not curvature' may be illustrated as follows: Take two circular wire rings, place one on another and dip them in

soap solution. While drawing them out of the solution separate them (keeping them parallel) so that a film stretches from one ring to the other but does not close either ring. A film shaped like the neck of an hour glass is produced (Fig. 7).

The reduction of surface tension by a solute may be illustrated as follows: Two slender dishes are filled with water and to one of them a trace of alkali or soap is added. A mixture of 2 parts chloroform and 3 parts rancid olive oil is sucked up into a pipette. The mouth of the pipette is held beneath the surface of the water and the mixture allowed to flow out slowly and drop off. The drops formed in the alkaline water are smaller than those in the pure water. This is due to the fact that the alkali acts on the fatty acid in the oil, forming soap, which lowers the surface tension. The

Fig. 7.



lessened surface tension supports a less weight of the mixture on the end of the pipette, and the drop falls before it reaches a large size.

It will be noted that the drops resting on the bottom in the pure water are approximately spherical in form because the high surface tension resists the action of gravity. However, the drops in the alkaline solution are more flattened because the surface tension is lessened and the resistance to the action of gravity is lessened. This difference is accentuated if, by holding the mouth of the pipette near the bottom, we cause drops of the same size to be formed in the two dishes. If we take a piece of linen thread .4 mm. in diameter, soak it in water and remove all air bubbles, then place it on one of the drops in the pure water, the high surface tension of the drop resists the force due to the weight of the thread, and the thread slides off of the drop. On repeating this experiment in the

alkaline water, the thread presses on and indents the drop, and if the surface tension of the drop is sufficiently lowered, the thread cuts it in two.

Instead of adding the alkali to the water, ROBERTSON soaked the thread in alkali. He used drops not over $\frac{1}{10}$ cc. in volume and submerged them not more than 5 mm. beneath the surface and soaked the thread in an alkaline solution $\frac{1}{10}$ — $\frac{1}{5}$ normal. In this way the surface tension was weakened just beneath the thread, and if the process is very rapid the drop might be cut in two without as much general flattening. Apparently my first attempts at repeating his experiment failed because the threads I used were not heavy enough to cut through the drop, for ROBERTSON says linen threads .2 mm. in diameter rarely cause division.

In an attempt to exclude the action of gravity ROBERTSON subsequently buoyed up the drop by saturating the lower layer of water with NaCl. He found that the alkaline thread sometimes cut through the drop, especially if the alkali was increased to a normal solution, but he adds: "The best method of performing the experiment is to lower an alkaline loop of thread into the water and draw it gently up through the drop." It should be noted that although the drop does not rest on a solid surface, the action of gravity is not entirely excluded, for the drop rests on a layer of NaCl solution of greater specific gravity than itself.

Surface movements due to local changes in tension may be illustrated as follows: If a glass plate is dipped into 95% alcohol, on removal it will be covered by a film of alcohol due to the adhesion of the alcohol to the glass. If a piece of cotton wet with water is held near a portion of the film, the surface tension will be increased, due to the localized increase in the water content of the alcohol. The surface of increased tension will contract, therefore each molecule in the surface film will move toward the center of the area. The friction on this surface layer of water will carry along layers beneath. Therefore all of the alcohol except that in close contact with the glass will be displaced toward the center of the area, where the alcohol is heaped up and the surface made convex. If the cotton is held near the surface of a spherical drop of alcohol, the surface currents are the same, but the molecules that moved to the center of the area turn downward (inward) and a vortex is formed. The surface of increased tension becomes more flattened due to the increased downward (inward) pressure of the surface film.

If the glass plate is wetted with water, and a rod wet with ether is brought near the center of the film, the ether vapor reduces the surface tension and the affected surface film spreads (as can be seen if powder is dusted over the surface). This spreading is caused by the pull of the surrounding surface film of high tension, which in turn is fastened to the glass at the periphery of the wet surface by adhesion. The water carried away from the center is temporarily banked up all around the edge of the affected area, and if the action is very violent, the center will become dry. This experiment is misinterpreted by ROBERTSON on page 705. If the ether is held near the surface of a spherical drop of water the affected area will be bulged out instead of sunken in, though this experiment is difficult to perform and observe in air.

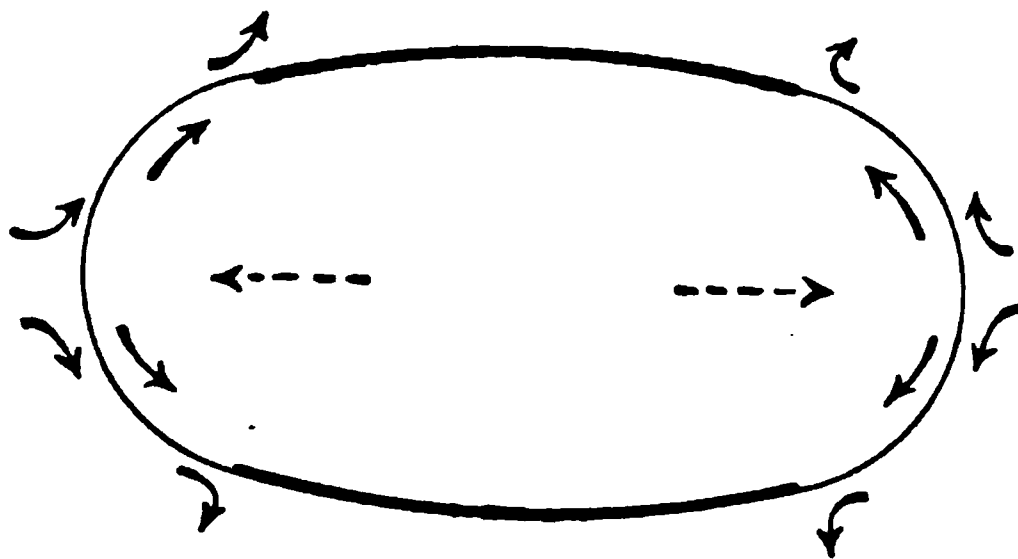
The pressure in a drop due to the tension of the surface film may be measured as follows: Water is sucked up into a vertical capillary tube and allowed to drop out until equilibrium is reached (a source of error arises in the final breaking off of too large a drop), and the height of the meniscus in the tube noted. A dish of water is then raised from beneath until the surface of the water in the dish touches the drop. The meniscus immediately falls. The meniscus is higher while the drop hangs from the lower end of the tube because the surface tension of the drop causes pressure within the drop which is transmitted up the tube and raises the meniscus. The pressure of a column of water equal in height to the difference in level of the meniscus represents the pressure within the drop.

It is not my ambition to exactly imitate cell division with a model, but an analysis of the following experiment may throw some light on what happens when a cell divides. A stender dish is half filled with distilled water and a funnel inserted so that the stem reaches the bottom. A saturated solution of pure NaCl in distilled water is poured very slowly into the funnel so that the salt solution comes to lie beneath the pure water without much mixing. About 1 cc. of the chloroform-oil mixture is poured in, so that it forms a drop suspended in the liquid. Two pipettes are filled with $\frac{1}{5}$ normal NaCl solution and quickly introduced into the water, and the alkali allowed to flow gently against opposite poles of the drop. If the alkali reaches both poles at the same time and in the same quantity, the drop elongates along the polar axis and usually constricts into an hour glass shape. It often divides into two at the constriction.

The conditions necessary for complete division are very limited, as is explained in the following analysis of the experiment:

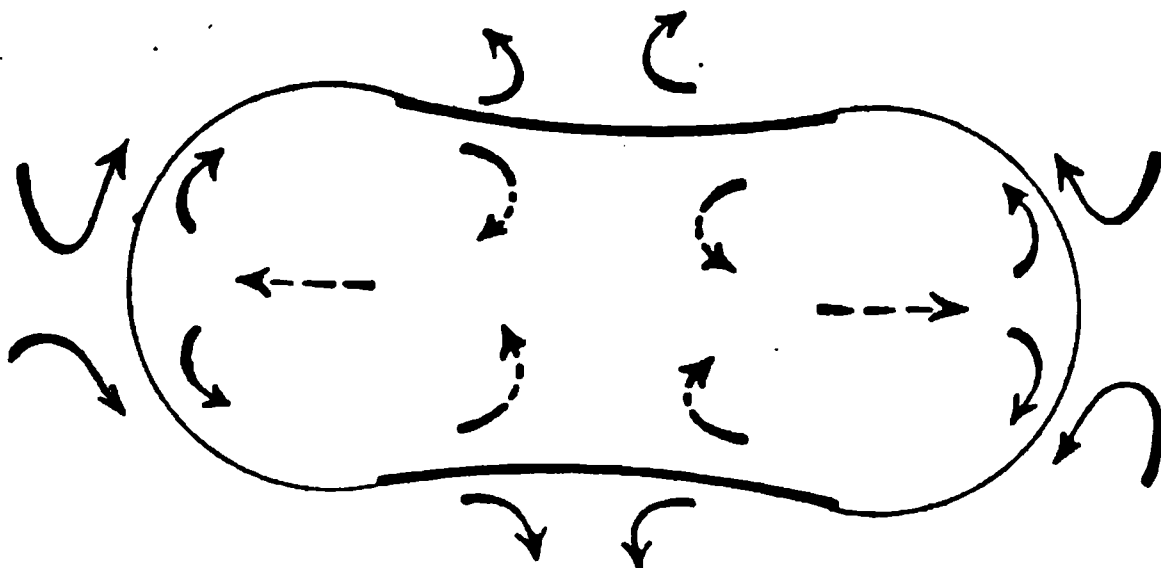
The alkali reduces the surface tension at the poles and the pressure inside the drop causes a bulging of these regions. In Fig. 8 the surface film of low tension is represented by a light line and that of high tension by a heavy line. At the same time the polar

Fig. 8.



surface films spread and the equatorial surface film contracts, producing vortex movements in the drop and in the medium as shown by the arrows (shown better in Fig. 9). These movements may be demonstrated by mixing a little soot with the oil and a few carmine grains with the water. The dashed arrows represent currents in the axis of the drop. The surface currents carry the alkali toward the

Fig. 9.

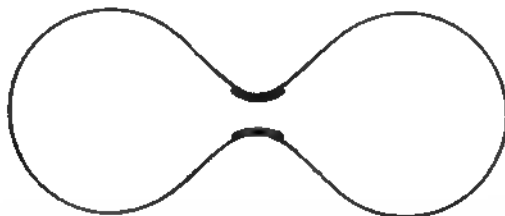


equator and enlarge the polar regions of reduced tension. The rapid fall in the internal pressure caused by the rapid enlargement of the surfaces of low tension allows a constriction of the equatorial region, as in Fig. 9. The equatorial surface is not concave (as ROBERTSON intimates) but remains >convex< until the meridional concaveness equals the equatorial convexness. When the surfaces of reduced tension have expanded until the area of high tension forms but a

narrow band around the constricted equator, this band acts like an isolated band of a tension equal to the tension of its surface minus the tension of the surface of the polar regions. The entire force of this residual tension is exerted toward the division of the drop (Fig. 10). However, the completion of the division takes time, and the band is rapidly reduced in width by the spreading of the alkaline surfaces. If the constriction is not completed before the alkali spreads over the whole drop, the drop returns to a spherical shape. Therefore it is unusual that a complete division of the drop occurs¹).

If a substance which increases the surface tension is liberated along an equatorial band, division will occur. However, a demonstration of this would be difficult for the following reasons: it would involve an increase in the sum total of surface tension, and since the surface is increased by division, a double increase of surface

Fig. 10.



energy would have to be brought about, and would involve a double amount of work to overcome the inertia of the system. This work would have to be expended in such a way as to increase the surface tension i. e., by locally decreasing the temperature, or the electrical polarization, or the concentration of a substance which decreases the surface tension, or by increasing the concentration of a substance which increases the surface tension. A great difference in temperature or pressure is necessary for a small difference in surface tension, and the mechanism of maintaining the equator at a lower temperature or higher pressure has not been devised. Whereas some dissolved substances slightly raise the surface tension of water, they affect the specific gravity in a more marked degree and inter-

¹) Failure to perform this experiment is due to lack of dexterity. The smaller the drop the (relatively) greater the surface tension but the greater is the dexterity required. Pipettes with capillary openings are more easily controlled, and the concentration of alkali may be increased to compensate for reduced quantity, but it should not be heavier than the salt solution.

ferre with the experiment. Dissolved substances which markedly raise the surface tension of liquids that could be used in a model, have not been found. If we start out with an oil drop in soapy water, the soap might be precipitated at the equator by a liberation of calcium, but the solid soap so produced would form a crust on the equatorial surface and interfere with its contraction. There remains the method of an equatorial decrease in electric polarization. A model on this principle has not been constructed. Electrical variations have, however, long been known to occur in living cells, and Miss HYDE has detected them in dividing eggs¹). I have discussed the possibility of cell division being due to an equatorial increase in surface tension, following decrease in electrical polarization due to increase in permeability, in previous papers²) (however, I can no longer assume protoplasm to have an invisible alveolar structure).

Now that the whole subject has been reviewed, a direct consideration of ROBERTSON's paper will be made. We have shown that in ROBERTSON's experiment the oil drop is divided by the gravitation of the thread which has been soaked in an alkaline solution. The alkali reduces the surface tension, thus reducing the resistance to division, and thus allows division by a thread of less weight than would otherwise be necessary.

ROBERTSON, on page 695, objects to the hypothesis that division can be brought about by an equatorial increase in surface tension, because the two drops resulting from division have a greater surface than the original drop, and if the surface tension is not decreased the potential energy of the surface is increased and work performed »without the introduction of any energy from without«. He forgets that work is done in increasing the surface tension of the equator. ROBERTSON's assertion (page 699) that regions which are convex to the medium have relatively high surface tension is only true when the surface tension of the medium is less than that of the liquid in question, at the surface of contact, as has been pointed out above.

ROBERTSON's argument on pages 699 and 700 falls flat when we note that M_1 and M_2 are not the same distance below the curved line *alrmd*.

¹) HYDE, Am. Journ. Physiol. Vol. 12. p. 241.

²) MCCLENDON, Am. Journ. Physiol. Vol. 27. p. 240; Biological Bulletin. Vol. 22. 1912. p. 158.

ROBERTSON criticises my illustration of a rubber balloon. I make no defence of this because it was never supposed to be an exact model of cell division.

ROBERTSON criticises my oil drop model of cell division. I have modified it as described above, to more nearly imitate cell division. However, I make no claims for this model, except that the dynamics of this model throw light on the dynamical possibilities in cell division.

ROBERTSON's illustration on page 705 of the effect of decrease of surface tension on the level of the surface, is not a general case, since the proximity of the glass plate interferes with the internal movements of the fluid. By increase in the thickness of the layer of water the result may be entirely changed.

ROBERTSON's illustration on page 705 of a boy enclosed within a football, trying to progress by pushing against the inside, without success, reminds me of my youthful experiment with the opposite result. While enclosed in a large barrel I caused it to progress very rapidly. Surface movements could cause the locomotion of an amoeba suspended in water by driving the water in the opposite direction, in the same way that a steamboat progresses.

As to ROBERTSON's hypothesis of the polar synthesis of nucleic acid in the daughter nuclei being causally related to cell division, I observed division of starfish eggs from which I had removed the nuclear material¹⁾.

Summary.

If a drop of liquid is suspended in a liquid medium, any area whose surface tension is reduced, spreads and protrudes, causing vortical currents, whereas any area whose surface tension is increased, contracts and becomes more flattened, causing a vortex in the opposite direction.

Division of a similar drop may be brought about by a condition in which an equatorial band has a higher surface tension than the remainder of the surface.

ROBERTSON came to opposite conclusions, but five fallacies in his argument are pointed out above.

¹⁾ MCCLENDON, Arch. f. Entw.-Mech. Bd. 26. 1908. S. 662.

Zusammenfassung.

Wird ein Flüssigkeitstropfen in einem flüssigen Medium suspendiert, so breitet sich jeder Bezirk mit vermindelter Oberflächenspannung aus und treibt sich vor, indem er vortikale Strömungen hervorbringt. Dagegen zieht sich jeder Bezirk mit vermehrter Oberflächenspannung zusammen und flacht sich ab, unter Veranlassung eines Wirbels in der entgegengesetzten Richtung.

Teilung eines ähnlichen Tropfens kann durch Verhältnisse zustande kommen, bei denen ein äquatorial gelegener Streifen höhere Oberflächenspannung besitzt, als die übrige Oberfläche.

ROBERTSON kam zu entgegengesetzten Folgerungen — im Vorstehenden sind aber fünf unrichtige Punkte in seiner Beweisführung aufgewiesen worden.

(Übersetzt von W. Gebhardt.)

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MIT BESONDERER BERÜCKSICHTIGUNG DES MENSCHEN,
FÜR STUDIERENDE, ÄRZTE UND ZÜCHTER

VON

Dr. LUDWIG PLATE

PROFESSOR DER ZOOLOGIE UND DIREKTOR DES ZOOLOGISCHEN
INSTITUTS UND DES PHYLETISCHEN MUSEUMS DER UNIVERSITÄT JENA

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Nature.

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Preparation of Material for Histology and Embryology, with an Appendix on the Arteries and Veins in a Thirty Millimeter Pig Embryo

J. J. MCLENDON

From the Anatomical Department of Cornell University Medical College, New York

REPRINTED FROM THE ANATOMICAL RECORD, Vol. 7
No. 2, February, 1914



PREPARATION OF MATERIAL FOR HISTOLOGY AND EMBRYOLOGY, WITH AN APPENDIX ON THE ARTERIES AND VEINS IN A THIRTY MILLIMETER PIG EMBRYO

J. F. McCLENDON

From the Anatomical Department of Cornell University Medical College, New York

THREE FIGURES

The essential of a good course in histology or embryology is good material. Fresh human material should never be allowed to go to waste, but it may be at times very inconvenient to put it up in a variety of fancy fixing fluids.

Perhaps the best general cytoplasmic fixer is formalin of 10 to 20 per cent (4 to 8 per cent formaldehyde). If material so fixed is not soaked too long in alcohol of high concentration, it may be used as fresh tissue in special technique to show fats or mitochondria. In fact the formaldehyde alone makes unsaturated fats and lipoids less soluble in clearing fluids. On the other hand, if the washing in water is omitted, the structure of resting nuclei is well enough preserved for ordinary purposes.

Commercial formalin contains formic acid, which, although developing a more beautiful nuclear structure, may begin to cytolyse the more delicate cells before they are sufficiently fixed by the formaldehyde. This is especially noticeable in erythrocytes—haemolysis, or escape of haemoglobin, occurring in parts of the tissue. Furthermore, acids swell fresh white fibrous tissue. It seems worth while, therefore, to neutralize the formol, and this may easily be done by adding slack lime (CaCO_3) or magnesia, and filtering.

Doctor Ferguson first called my attention to the fact that kidney swells in many fixing fluids, whereas it is commonly supposed

that the majority of tissues shrink a little. Death of isolated cells as seen under the microscope may be accompanied by swelling (cytolysis) or contraction. In every case, an increase in permeability to some substances occurs, but I found that during the early stages of cytolysis of the sea urchin's egg, it remains very impermeable to salts. Dead animal or plant membranes are more permeable to water than to dissolved substances, but apparently some living cells are impermeable to water. The *Fundulus* egg, if transferred from sea water to distilled water, does not burst, though it is certainly not capable of resisting the enormous osmotic pressure of its internal salts. Since I found this egg to be impermeable to salts, it must also be impermeable to water (it is permeable to kations, but for every kation that comes out, the electrical equivalent must go in). If such a cell became, on death, permeable to water, the osmotic pressure of its internal dissolved substances might cause it to swell. If a *Paramoecium* be killed by an ordinary fixing fluid, even though it be hypertonic, the protoplasm first coagulates, then the whole animal swells a little. This may be what happens to some tissue cells, and I found that it is not always prevented by the addition of 0.9 per cent sodium chloride to the fixing fluid. Therefore I supposed the swelling due to the osmotic pressure of some contained substance of large molecule, and experimented with the addition of cane sugar to neutral formol. By this means the cytolysis of adult convoluted nephric tubule cells is prevented, and the general fixation is good except that some nuclei may be slightly shrunken. This fluid may be used for all adult tissues and embryos, and is easily prepared as follows:

Formol.....	100-200 cc.
Cane sugar.....	20-40 grams
Slack lime (CaCO_3) or magnesia.....	about 1 gram
Water to make 1 liter.	

If the shrinkage of a few nuclei is very objectionable use only 20 grams of sugar. This fluid has the advantage that tissues and embryos float in it and therefore do not become distorted.

If the whole kidney of a fetus be fixed in the above mixture or any other fixing fluid, the cells of the convoluted tubules will

swell until they fill the lumen. This brings us to a well known point that is often neglected. Tissues should be cut into as thin slices or pieces as is practicable and the cells not injured in the cutting. Fetal tissues are especially delicate. They should be cut with a very sharp thin blade and lifted on the blade into the fixing fluid.

Many workers object to formalin because it "causes" a homogeneous appearance to protoplasm. The ultra microscope has shown that, aside from evident granules, living protoplasm is homogeneous, contrary to Bütschli and others. There are persons who now accept formalin for cytoplasmic fixation but say that it "does not fix nuclei well." Some structures may be seen in living nuclei. I have studied many nuclei with high powers and with the ultra microscope, yet I cannot decide what form of fixation corresponds most closely to the living structure. Both cytoplasm and nucleus of a living erythrocyte of a frog is homogeneous when examined in serum or uncoagulated plasma with the ultra microscope. Sooner or later bright points or clouds appear on or in the nucleus, but this is usually associated with change of nuclear form and is evidently due to injury.

Formaldehyde not only does not coagulate protoplasm but renders it more difficult to coagulate. It also makes lipoids less soluble in clearing fluids. However, I find an after-treatment with Müller's fluid or some other oxidising fluid necessary for the preservation of lipoids, the amount of oxidation necessary depending on whether mitochondria, myelin or fats are studied.

Ordinary staining depends on the fact that all protoplasm treated with acid, stains with acid dyes, whereas certain parts take also basic dyes. Many staining solutions contain free acid, but tissues stain more quickly if they are previously treated with acid. For this reason we put everything into the formol mixture and after a few hours transfer part of it to Bouin's fluid. This tissue is finally stained on the slide in haematin and eosin. The alum haematin lake is usually so strong that it stains in three minutes, but the eosin is so much diluted that twelve hours are required to stain and in this time smooth muscle stains less intensely than white fibrous tissue. The acid in

Bouin's fluid causes the tissue to stain more brilliantly but if the fresh tissue is put into Bouin's fluid the blood in some of the vessels will be laked. Part of the material is transferred from the formol mixture to Müller's fluid and subsequently stained with iron hematoxylin to show the lipoids (mitochondria, etc.).

Ordinarily, the student is shown two dimensions of a piece of tissue or embryo, and left to imagine the third. Though whole mounts of chick embryos are handed out, cleared pig embryos, and blocks or thick sections of certain tissues are even more useful. For a solid mount, the object should be placed in a dish of balsam or damar dissolved in benzol and protected from dust until it evaporates down to sirupy consistency, then mounted in the usual way. By this means the necessity of rings or other supports to the cover glass is avoided, and drying out or great shrinkage prevented.

All of the solid mounts turn yellow with age, but a number of highly refractive fluids may be obtained that are colorless. These are listed, with their refractive indices, in Landolt-Bornstein; Behren's Tabellen; and Lee's Vade Mecum. The higher the refractive index the better, for if in any case a lower index is desired, this may be obtained by the addition of paraffin oil or xylol (or water in case of aqueous media). It may be noted here that, whereas the process of clearing in a mixture of oil of wintergreen (Gaultheria) and benzyl benzoate has been patented in Germany and is widely known under the name of the patentee, wintergreen was first used by Stieda in 1866, and the synthetic oil (methyl salicylate) recommended by Guéguen in 1898, and is noted in various books on technique.

Methyl salicylate is permanently colorless, and comparatively inexpensive, and ideal for a fluid mount. If rings are cemented on slides with shellac or liquid glue and allowed to dry, they are not loosened by the oil. Paper rings soaked in shellac or glue will do, but rings may be cut from lead pipe with an ordinary saw or a bone saw if the proper size of glass rings are not at hand. The shellac must be dry before adding the oil, which must be free from alcohol. I prefer glue.

If the tissue is hardened in alcohol, thick sections may be cut free-hand. Thick sections are often better unstained, especially

if injected, and much detail may be made out by partly closing the diaphragm of the microscope. If stained with very dilute haematin containing much acid, connective tissue is colorless and cytoplasm nearly so, whereas nuclei may be readily distinguished. In this way blood vessels and glands in areolar tissue are caused to stand out sharply.

Whole mounts and thick slices are especially useful in embryology, and are a necessity unless one is contented with teaching the third dimension with models. The larger the embryo, the more attention must be paid to the clearing medium in order to distinguish internal structures. Methyl salicylate is admirable for pig embryos of all sizes and even for small fetuses. I found ethyl salicylate to be as good if not better, but it is more expensive. Canada balsam has about the same refractive index ($n_D = 1.535$) as methyl salicylate ($n_D = 1.536$), but darkens with age.

Embryos may be placed directly from absolute alcohol, benzol, xylol, toluol or chloroform into methyl salicylate, but in order to obtain the proper refractive index, the preliminary fluid must all be removed. This may be evaporated, or washed out with more wintergreen. Benzol is to be recommended because it is cheapest and evaporates out most easily. The evaporation may be hastened by an air pump, which also removes any air bubbles that may get into the specimen. These bubbles expand and are absorbed after the pump is disconnected, or by successive pumpings. An ordinary air pump will cause the benzol and air to boil out. A water-suction air pump (aspirator) will suffice but a float valve and safety bottle should be interposed between the pump and the specimen to prevent the back flow of water. An exhaustible desiccator is convenient for holding large embryos while they are being pumped out. If the cover is well ground, the oil will seal it sufficiently, and vaseline should not be used.

Most of the internal organs may be distinguished in unstained embryos by cutting down the light. The individual cells of mesenchyme, cartilage and blood may be seen; the cellular structure of the neural tube is indicated by radial striations and the

larger nerves appear as bundles of fibers. Some organs in smaller embryos are made more distinct by staining with very dilute alum haematin containing a large amount of acid.

Even in quite small embryos, many of the blood vessels may be traced by the blood cells, and the large empty veins followed as cavities. However, with the smaller vessels this becomes more laborious than serial sections. On the other hand, the injection of small embryos for class use means quite an outlay of time. Therefore, it seemed necessary to find some way to fix the haemoglobin, and keep the vessels full, in order to distinguish the vessels by the color of the blood. I found that *the same method that prevents the cytolysis of nephric tubule cells prevents haemolysis*.

Living embryos are obtained, the amnion opened, the placenta squeezed to force the blood into the embryo, and the umbilicus tied or clamped. Artery clamps are too strong and pinch off the cord. (I made clamps out of wire (lower part of fig. 1) in order to avoid tying so many cords at the slaughter house. The clamp may be removed in half an hour and used again.) The embryo is dropped into the neutral-formol-sugar mixture described above, and left until thoroughly fixed. In case of a fetus, part of the skin should be torn off after the superficial blood vessels are fixed, to insure penetration of the formaldehyde. A hole may be made in the skull by slicing off a small piece tangentially or by a sagittal cut near (to the right of) the median plane. Large fetuses, unless skinned completely, will have to be scraped to remove the pigment layer.

Transfer the specimen after washing, or directly, from the fixing fluid to alcohol of about 70 per cent. After they have hardened in 80 or 95 per cent alcohol it is well to split the large specimens by a sagittal cut a little to the right of the median plane with a very thin bladed knife. The dehydration with higher alcohols should be slow enough to prevent shriveling.

By this method the blood retains its color, and although it does not take the place of injection, it is a great help to the student. I have inserted three figures to show what can be seen in such specimens.

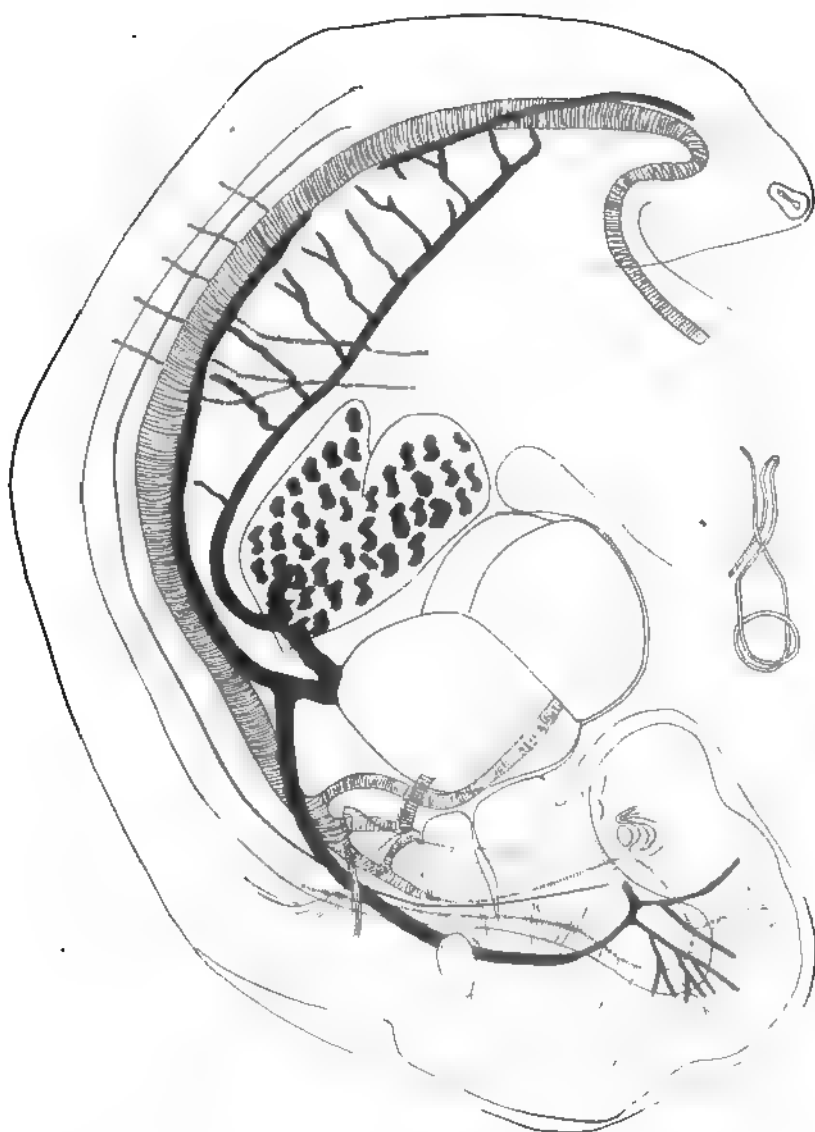


Fig. 1 Left half of a pig embryo 7 mm. long after clearing. The veins are black, the arteries cross-striated, the 5th, 7th, 8th, 10th and 11th cranial nerves are longitudinally striated, the notochord is represented by a heavy line and the fore gut by a dashed line. The sinuous line ventral to the embryo represents a wire (partly open) clamp used in clamping the umbilicus.

Figure 1 represents a pig embryo about 8 mm. long. The nerve tube, fore gut, mesonephros, liver, heart, eye and ear are clearly seen. The arterial system and part of the cardinals and subcardinals can be distinguished. The notochord is distinct, and the 5th, 7th, 8th, 10th, and 11th cranial nerve roots can be made out. Figures 2 and 3 are described in the appendix.

I have prepared hundreds of pig embryos and fetuses in this way, and also injected many with india ink and cleared them in wintergreen oil. A completely injected fetus can only be studied in comparatively thin (freehand) sections. Various degrees of partial injection are very useful to show the larger vessels, but these may be seen in the uninjected fetuses. The left side of an uninjected fetus which has been cleaved a little to the right of the median plane, will show the general circulation, except in the liver. The larger vessels in the liver may be seen by removing the lateral portions and passing a strong light through the remainder (an arc light is excellent), or the liver may be removed and cut into slices. In injected specimens the liver is hopeless.

I washed with alcohol the blood out of the vessels of a fetus 4 inches long and cleared it in wintergreen oil, then injected it with mercury. This method has the advantage that the extent of the injection may be watched and controlled.

The injection may be limited by using a coarse granular pigment that will not go into the capillaries. A gelatine mass is not absolutely necessary to hold the pigment. A light colored opaque pigment has the advantage that it may be seen by transmitted or reflected light.

The arteries may be injected and the haemoglobin fixed in the veins, giving handsome specimens. If it is desired to show only the injection, no formalin should be used. Much of the haemoglobin may be dissolved out by putting the fresh specimen into weak alcohol or alcohol and acetic acid. All of the haemoglobin may be removed with dilute acetic acid provided an injection is used that is not affected by this acid.

APPENDIX


ON THE ARTERIES AND VEINS IN A 30 MM. PIG EMBRYO

The method of fixing the haemoglobin and clearing in winter-green oil to show the course of the vessels has been especially successful in case of pig embryos of about 30 mm. length. Figures 2 and 3 show the larger vessels of the median plane and left side of one of them. The courses of most of the vessels approach the type of the adult pig and show distinctions in topography from those in man. The common carotid artery and (right) innominate artery arise from a common trunk, the brachio-cephalic artery. The posterior inferior cerebellar artery arises from the basilar instead of from the vertebral.

Notwithstanding the great development of the vena cava, the left posterior cardinal is of considerable size. The right cardinal (not figured) is smaller. The thoraco-epigastric vein is divided into two parts, one of which drains anteriorly into the internal mammary.

The vessels of the limbs could not be completely followed, but enough was seen to demonstrate that they differ very much from those in the adult.

Besides the vessels, the mouth cavity, brain, eye, endolymphatic labyrinth, lungs, mesonephros, kidney, testis and penis are outlined in the figures.



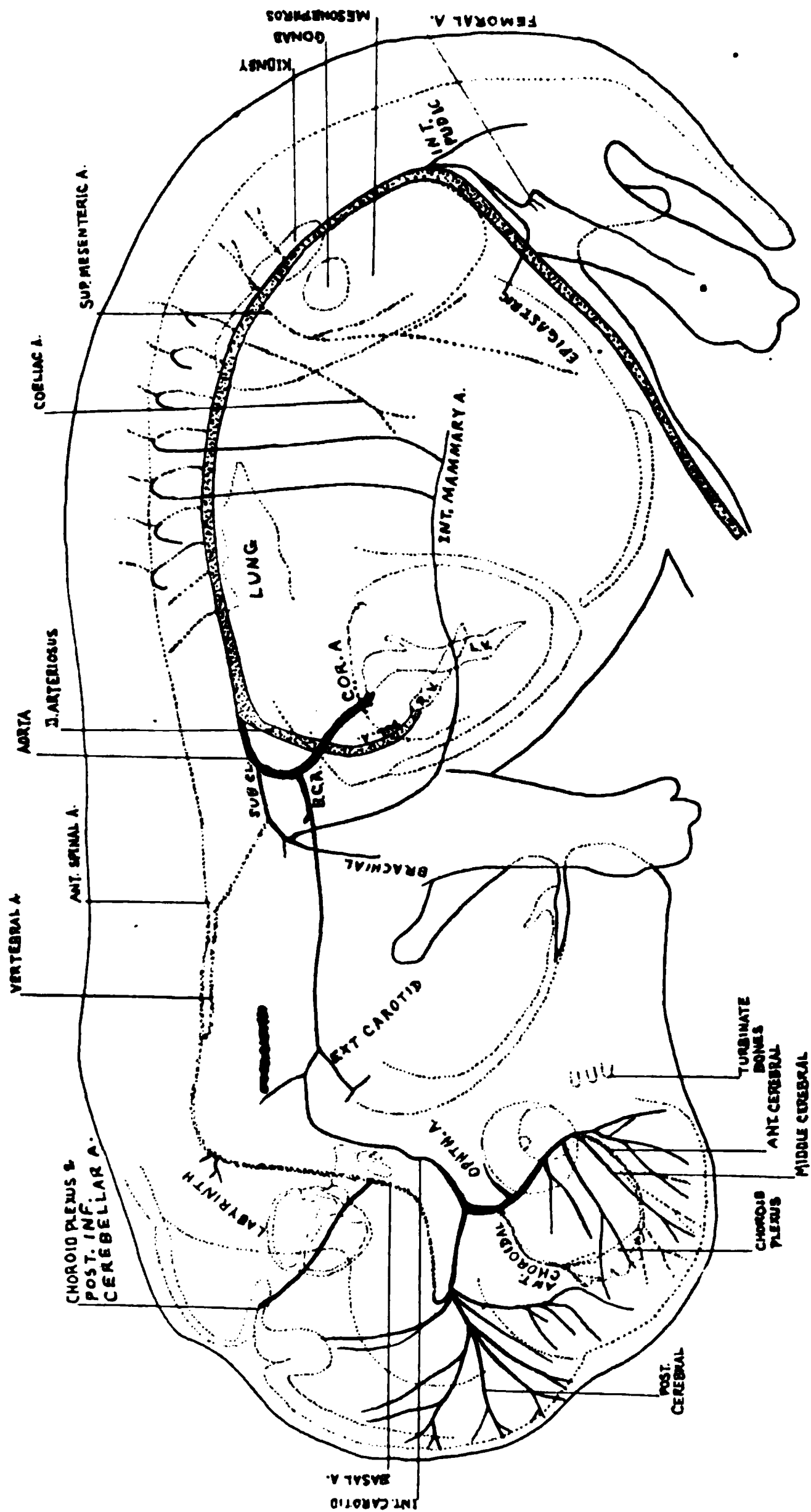


Fig. 2 The arteries of median plane and left side of a pig embryo 27 mm. long after clearing. The arteries farthest from the observer are stippled. The musculo-fibrous coats are not represented, and some of the arteries are so contracted as to almost obliterate the lumen. *B.C.A.*, brachio-cephalic artery; *COR.A.*, coronary; *SUBCL.*, subclavian; *PUL.A.*, pulmonary; *OPHTH.A.*, ophthalmic; *LV.,RV.*, cavities of left and right ventricles.

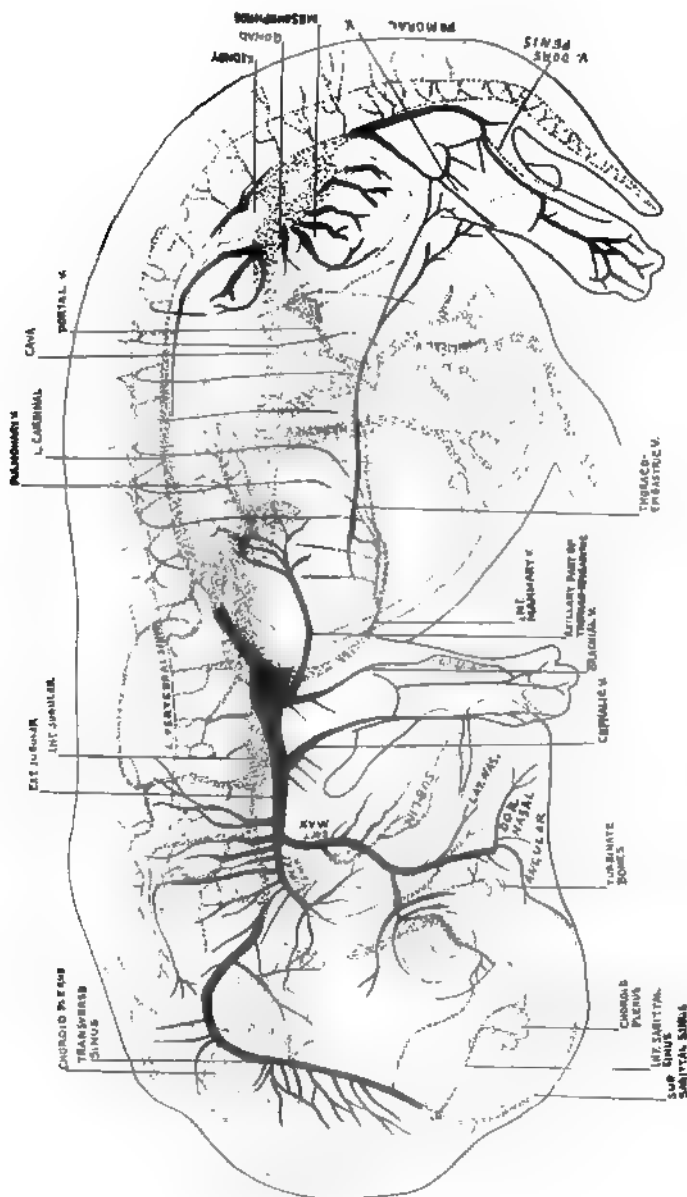


Fig. 3 Veins of median plane and left side of a pig embryo 27 mm. long after clearing. Each vein is represented by its lumen only, but some have been purposely made smaller for clearness in the figure. The representation of the veins between the mesonephroi is somewhat diagrammatic.



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the Absorption of Water through the Skin of a Frog

by

J. F. Mc Clendon

Abdruck aus: Internat. Zeitschrift für physik.-chem. Biologie. I. Band, 3. u. 4. Heft

Leipzig und Berlin

Wilhelm Engelmann

1914

1

On the Absorption of Water through the Skin of a Frog.

By

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University of Minnesota, Minneapolis.

(Contribution from the Anatomical Department of Cornell University
Medical College.)

(Eingegangen am 4. Februar 1914.)

The work of OVERTON seemed to show very clearly that the whole frog, or the excised muscle, when placed in water or a hypotonic solution, absorbs water, and that the driving force of this absorption is the osmotic pressure of the tissue and cell fluids. It was rather surprising, therefore, that MARTIN FISCHER should imagine that the driving force of this absorption is the swelling of colloids, and to construct on this hypothesis theories of edema and nephritis. I do not wish to consider either edema or nephritis, but merely to consider whether any quantitative relations of the absorption of water by the frog or through the frog's skin are inexplicable on the theory of osmotic pressure.

FISCHER ligated the leg of the frog at the knee so as to cut off the circulation of blood and lymph, and so long as the leg remained in water, it absorbed water. Obviously it made no difference whether the leg was amputated or remained attached to a live frog. My explanation of this is that the leg, and all other parts, of the frog is constantly absorbing water while immersed, but that this water is constantly being eliminated by the kidneys so that the leg retains its normal water content. But as soon as communication with the kidneys is interrupted by a ligature at the knee (thus stopping the blood and lymph flow) the water absorbed begins to accumulate in the leg. The same is probably true of the excised muscle, eyeball or any other part, while living, and perhaps for some time after death of the cells.

It was not practicable to measure the volume of blood flowing to the leg and subtract it from the volume of blood (and lymph) leaving the leg in order to see whether the leg with a circulation absorbs water at the same rate as the leg that is ligated. However, by making the

assumption that water is absorbed through all regions of the skin of the immersed frog at the same rate per unit area, a method of experimentation was found.

The skin area of a leopard frog (*Rana pipiens*) exclusive of the head was measured by pasting pieces of waxed cross section paper over it and then computing the number of squares on the paper. This may seem a crude method but it was the most accurate that was found. By this means it was determined that the ratio of the whole skin area (exclusive of the head) to the area of the two hind legs (and feet) below the knees, was 3,495. In a wood frog (*Rana sylvatica*) this was found to be 3,497. Since the errors of measurement are probably too great to make the third decimal place of any use, we will consider this ratio as 3,5.

Two leopard frogs of the same weight were selected. One was weighed and then fixed in an apparatus so that the urine was collected, and the frog kept immersed up to the head in water. It could drink no water and it breathed a damp atmosphere, so that there could be no evaporation from the lungs. The two legs of the other leopard frog were ligated just above the knee, amputated immediately above the ligature and weighed, and then immersed in water. At the end of twenty hours the frog (+ urine) was weighed and the two legs were weighed. Taking the average results of three experiments the frog had increased 11 grams, the legs had increased 1,1 gram. The ratio is 10 as compared with 3,5 for the ratio of skin areas. In other words, the absorption of water through the skin of the normal frog was nearly three times as rapid as the absorption of water through the skin of the excised legs per unit area. On the osmotic theory this may be explained by the fact that the osmotic pressure throughout the normal frog was maintained equal to a 0,7% solution of NaCl by the action of the circulation and kidneys, whereas, the osmotic pressure in the skin of the amputated leg was rapidly lowered by the mixture of the first water absorbed with its fluids, and hence the rate of absorption of water was rapidly reduced.

In an experiment with wood frogs lasting five hours, the frog increased in weight 0,67 grams and the two hind legs 0,15 grams. In this case the ratio was 4,5 or not very much more than the ratio of skin areas, 3,5. It seems probable that if the time had been very short, the ratio would have been the same, but owing to errors concerned with weighing, this was not attempted, as it was feared that the increase in weight would not be very much larger than the errors. It should be noted that every effort was made to reduce errors — the skin was

wiped in the same manner each time and the frogs and legs were weighed in closed glass vessels to prevent evaporation.

The effect of circulation of the contained fluids in the leg was shown in the following manner: It was determined that in 0,7% NaCl the leg of a wood frog neither gained nor lost weight.

The two legs of a wood frog were amputated above the knee and one was ligated just below the amputation. The other was carefully skinned and the leg taken out without injuring the skin. The skin was then filled with 0,7% NaCl solution and ligated at the same level as the other leg and the results given in the following table:

Hrs.	Increase in weight in grams	
	leg	skin
1	0,7	0,07
3	0,155	0,21
6	0,22	0,47

It is seen that the absorption of water by the leg, and the skin filled with salt solution, continued to be the same for one hour, during which time the water absorbed was probably all held in the skin itself. After this, however, the skin filled with NaCl solution absorbed water faster. The various membranes and colloidal "gels" in the frogs leg retarded the inward diffusion of the water, so that it was retained in the superficial layers and so diluted the fluids as to reduce the osmotic pressure and retard further diffusion. The water absorbed into the NaCl solution was rapidly distributed by convection currents and free diffusion, throughout the whole solution, and the osmotic pressure was but slowly lowered.

Since the skin of the frog is electrically polarized (being negative on the outer surface) and since electric polarization has been claimed to cause polarized or one-sided diffusion, experiments were set up to compare the rate of osmosis through frogs skin in opposite directions.

The legs of a wood frog were skinned and one of the skins turned inside out. The two skins were filled with 0,7% NaCl solution and immersed in water. If there was any difference in the increase in weight of the two skins it was too small to be detected.

In a second experiment a piece of wood frog's skin was stretched tight as a diaphragm separating water from 0,7% NaCl solution and the rate of osmosis determined. Water was first placed on the "outside" and 0,7% NaCl on the "inside". After half an hour water was placed on the "inside" and 0.7% NaCl on the "outside" and the osmosis

continued another half hour. No difference in the rate of osmosis in the two directions was detected.

That electric polarization and other factors that are dependent on the life of the cells in the skin affect diffusion of some substances I do not doubt, but nearly, if not quite 95% of the osmosis of water in these experiments is fully accounted for by the osmotic pressure of the fluids in and between the cells of the tissues.

Contrary to FISCHER, the effect of stoppage of the circulation is to decrease rather than increase the absorption of water. Only after the tissue has long been dead does an acidosis, autolysis or increase in permeability increase the absorption of water.

Summary.

The absorption of water by a frog's leg in which the circulation has been stopped is the result of the osmotic pressure of the fluids in and between the cells of the tissues.

Zusammenfassung.

Die Wasserabsorption eines Froschschenkels, in welchem die Zirkulation gehemmt wurde, ist das Resultat des osmotischen Druckes der Flüssigkeiten in und zwischen den Gewebezellen.

Postscript: This paper was read Apr. 16, 1913: Proc. Soc. Experimental Biology and Medicine, X, 125. Since then a paper by S. S. Maxwell: Amer. Journ. Physiol., XXXII, 286, appeared, on the same subject which does not, however, disagree with my conclusions. I found the freezing point of the blood serum of the leopard frog (*Rana pipiens*) lower than that of a 0.65% NaCl solution, and the same was found true of *Rana esculenta* by BOTAZZI.

121.

On the Electric Charge of the Protoplasm and other Substances in Living Cells

by

J. F. McClendon

With one figure in text

Sonderabdruck aus: »Internat. Zeitschrift für physik.-chem. Biologie.« I. Band, 3. u. 4. Heft

Leipzig und Berlin

Wilhelm Engelmann

1914

On the Electric Charge of the Protoplasm and other Substances in Living Cells.

By

J. F. McClendon,

University of Minnesota, Minneapolis, U.S.A.

(Contribution from the Anatomical Department of Cornell University Medical College.)

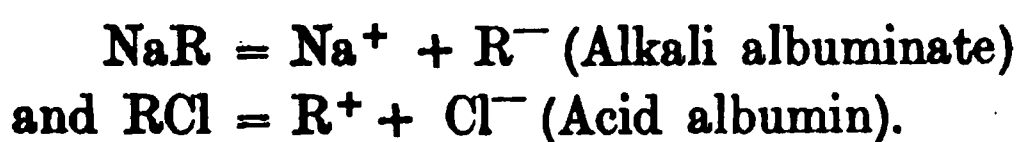
With 1 figure in text.

(Eingegangen am 3. Februar 1914.)

A number of investigators have observed that when an electric current is passed through a suspension of animal cells, it carries them passively toward the anode, indicating that the cells are charged negatively. This may be explained by the hypothesis that the plasma membrane of the cell is permeable only to kations, so that after the loss of some kations by diffusion, the excess of contained anions gives the cell a negative charge. Another hypothesis is that all of the protoplasm is charged negatively.

Electric currents were passed through animal and plant cells, and many substances of or in the protoplasm migrated toward the anode, showing that they were charged negatively. It appeared, also, that the denser substances, such as chromatin granules, yolk platelets and mitotic figures migrated faster than the other substances. Though this tended toward the belief that all of the cell contents, except water, are charged negatively, the slowness with which hyaline parts of the protoplasm of some cells migrated prevented a determination of their electric charge. The object of the present paper is to speculate on the cause of this negativity of protoplasm.

It is generally recognized that proteids, such as egg albumin, are anodic (or negative) in alkaline solution and kathodic (or positive) in acid solution, because they are amphoteric electrolytes and dissociate according to the formula:



We might postulate that the interior of the cell is alkaline and that the superficial protoplasm dissociates positive ions, such as Na^+ , into the surrounding medium, thus making the cell negative, or that it absorbs OH^- ions.

It has been commonly supposed that the reaction of the cells of the body is maintained constant by osmotic interchange with the blood, and the blood was formerly considered alkaline. But the blood has been shown to be neutral, and many cells appear to be impermeable to many substances that might affect their reaction. Furthermore, there seems to be no direct method of testing the reaction of the cell interior unless we rely on indicators. Those indicators taken up by living cells, as neutral red, are immediately bound up in some manner with colloids, and it seems unwise to regard them as infallible.

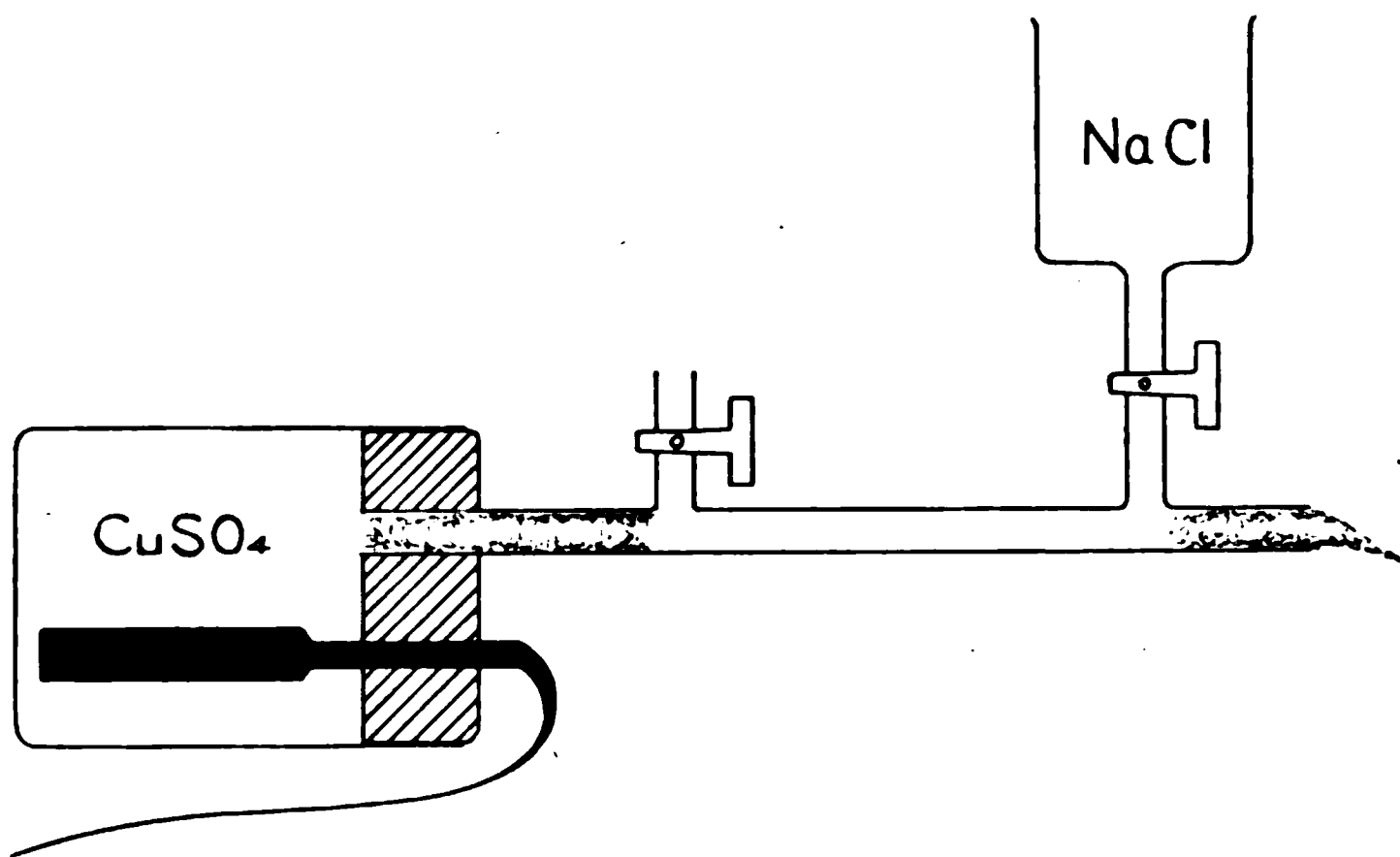


Fig. A.

One of the two electrodes used in passing the 116 volt direct current through cells placed between slide and cover glass, under the microscope. The non-polarizable electrode is connected with a glass tube filled with NaCl solution (usually about 1%) a plug of cotton retards diffusion between CuSO_4 solution and NaCl . Another plug of cotton closes the end of the tube and is pulled out to a point to communicate with the preparation under the microscope. By opening the two stops cocks any CuSO_4 which has diffused into the tube may be washed out, by a flow of solution *toward the electrode*. The amount of current may be changed by the concentration of salt in the solution.

If protoplasm is negative, because it is saturated with an alkaline solution, the supposition would easily follow that treatment with acid would make it positive, just as egg albumin is made positive. To test this I found plant cells that are permeable to acid while yet alive. But these cells are easily injured by the electric current, and the presence of the acid seemed to make them more sensitive to the electric current, so that they died before any appreciable amount of protoplasm had been displaced.

Although these experiments have failed in regard to observations on the living "protoplasm", interesting results were obtained on the electric convection of substances in the vacuoles of living plant cells. The vacuoles of some plant cells contain a colored substance, anthocyan in (colloidal?) solution.

Although very little chemical significance is attached to this term, anthocyan is an acid-alkali indicator. It has been suggested that in some cells, such as those of the nectar glands of *Vicia faba*, the anthocyan changes from blue to red and vice versa during the functional activity of the cell. These cells and also those of red beet and red cabbage are permeable to acids and alkalis, as indicated by the change in the color of the anthocyan. In cells of the red cabbage the anthocyan is normally colored red and is kathodic, but if ammonia is added it becomes blue-green and is anodic. This experiment is a very tedious one because the cells are easily injured by the current. Notwithstanding this disadvantage, I succeeded in 1910 in observing the movement of red anthocyan toward the kathode and blue-green anthocyan toward the anode in cells, before any signs of death or injury occurred. These experiments have recently been repeated many times to exclude any possibility of error. We may conclude, therefore, that the anthocyan is an amphoteric electrolyte and that its red color and kathodic migration in any cell denotes acidity of the cell sap in which it is dissolved.

If the protoplasm behaves as the anthocyan, many plant cells are probably acid and kathodic, since the anthocyan is red. The protoplasm of cells in growing root tips appears to be anodic. The protoplasm of old cells forms a thin layer that sticks tightly against the cell wall and cannot easily be dislodged by the electric current. This cell wall also interferes with the interpretation of results on the electric convection of the whole cell, since the whole cell would have the charge of the superficial layer, in this case, a cellulose cell wall. THORNTON¹ observed that plant cells differed from animal cells in being usually kathodic. Some of these cells were motile and might have shown galvanotropism, but others were not. Even non-motile naked cells might be kathodic on account of adsorption of positive ions. HÖBER² observed that the addition of small amounts of H^+ , Ag^+ , Cu^{++} , or Fe^{+++} , made erythrocytes and yeast kathodic, and very probably the heavy metals were adsorbed. HÖBER concludes³ that CO_2 makes erythro-

¹ THORNTON, Proc. Royal Soc., B., Vol. 82, p. 638, 1910.

² Physikalische Chemie der Zelle im Gewebe, S. 388, 1911.

³ Ibid. p. 495.

cytes kathodic by increasing their permeability to anions since they become kathodic, only in an anion-poor solution (2% NaCl) and not in an anion-rich solution (8% NaCl). All of these observations show that in such cases we should not consider the electric convection of the whole cell as indicating the electric charge of the whole protoplasm, but only of the superficial layer of the cell or cell wall.

Summary.

The anthocyan in the vacuoles of living cells of red cabbage is red and is kathodic (electrically positive), if alkali be added to the medium the anthocyan becomes blue and anodic (electrically negative) before the death of the cell. Therefore, the anthocyan seems to be an amphoteric electrolyte like egg albumin.

It seems possible that the reason that protoplasm is anodic is because it is alkaline, but this is not demonstrated.

Zusammenfassung.

Das Anthocyan in den Vakuolen der lebenden Zellen von Rotkraut ist rot und ist kathodisch (elektrisch positiv); wenn man Alkali zu dem Medium fügt, wird das Anthocyan vor dem Tode der Zellen blau und anodisch (elektrisch negativ). Daher scheint das Anthocyan ein amphoterer Elektrolyt, wie Eiereiweiß, zu sein.

Es scheint möglich, daß der Grund, weshalb Protoplasma anodisch ist, darin liegt, daß es alkalisch ist, aber das ist nicht bewiesen worden.

11

the Nature and Formation of the Fertilization Membrane of the Echinoderm Egg

by

J. F. McClendon

With one figure in text

Abdruck aus: »Internat. Zeitschrift für physik.-chem. Biologie.« I. Band, 3. u. 4. Heft

Leipzig und Berlin

Wilhelm Engelmann

1914

On the Nature and Formation of the Fertilization Membrane of the Echinoderm Egg.

By

J. F. McClendon,

University of Minnesota, Minneapolis, U.S.A.

(Contribution from the Anatomical Department of the Cornell University Medical College and the U.S. Bureau of Fisheries Laboratories at Wood Hole, Mass.)

With 1 figure in text.

(Eingegangen am 3. Februar, 1914.)

Many investigators have observed the formation of a sharp line around the echinoderm egg shortly after fertilization. This line is usually supposed to be the optical section of a membrane, the so-called "fertilization membrane". Some observers, notably HARVEY¹ suppose the membrane to be present before fertilization, but to be so closely applied to the egg as to be almost invisible, and after fertilization to be raised from the surface of the egg by the accumulation of a perivitelline substance between the membrane and the egg. LOEB supposed this perivitelline substance to contain a colloid which absorbs water and swells, this pushing out the fertilization membrane. He describes the perivitelline substance as exuding from the egg in droplets, so that one might consider it a colloidal solution. However, it seems to be a "gel" since it assumes a striated appearance, when put under tension; and the egg does not sink or rise in it but maintains a fixed position, as I have determined by observing the egg horizontally in a specially constructed chamber.

KRRE² attempted to disprove the existence of a fertilization membrane. According to his observations, the unfertilized egg is surrounded by a hyaline layer which he calls the vitelline membrane. He claims that fertilization results in a swelling or increase in thickness of this vitelline membrane, and that what appears to be the fertilization

¹ Jour. Exper. Zool., VIII, p. 365, 1910.

² Science, n. s. 36, p. 562, 1912.

membrane is really the outer surface of a thick membrane, the swollen vitelline membrane. It appears to me that KITE is merely applying new terms to structures which were considered by previous observers, unless he can show that the swollen vitelline membrane is homogeneous. If the outer surface film of this swollen vitelline membrane is more rigid than its interior, then it represents a distinct structure and we are justified in applying the term fertilization membrane to it. The following evidence seems to show that the fertilization membrane is really a structure.

1. In normal development, the fertilization membrane does not sink into the cleavage furrows as the hyaline plasma layer and perivitelline substance does (*Arbacia*).

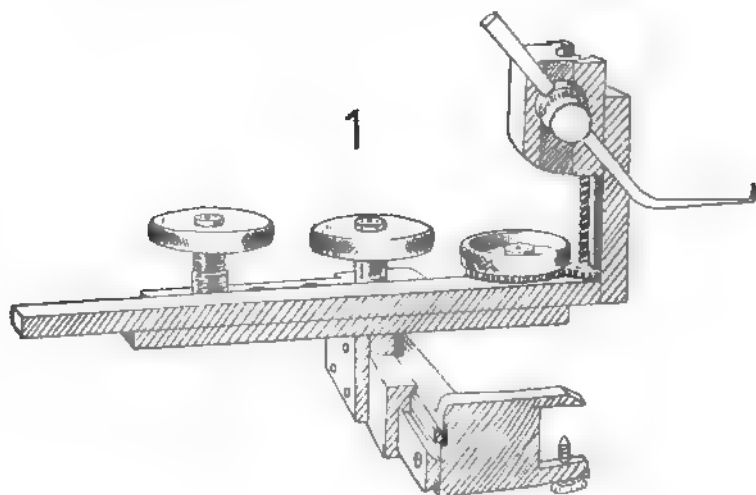


Fig. 1.

„Mechanical hand” used to hold a glass needle or pipette in fine work under the microscope (first described by McClendon; *Biological Bulletin*, XII, p. 141, 1907). The glass needle shows two bends but usually a glass rod held horizontally and with the tip drawn vertically to a point was used. The egg was in a very thin hanging drop in a damp chamber. The apparatus may be clamped on either side of the microscope stage, preferably on the right, or two may be used simultaneously. Made by Spencer Lens Co., Buffalo, N.Y.

2. Cilia develop before the disappearance of the fertilization membrane and beat actively in the space beneath the fertilization membrane (*Arbacia* and *Asterias*).

3. I have manipulated the fertilization membrane of the *Arbacia* egg with glass needles and punctured it and removed it from the egg and it appears to offer more resistance than the perivitelline substance to the needle (see fig. 1). The egg may be forced out of the fertilization

membrane by means of the electric current¹ and the membrane remains spherical and of large size as though it had considerable rigidity².

It seems clear that the old view of the existence of a fertilization membrane is pretty near the truth, and we will now consider its mode of origin.

The egg of the sea urchin, starfish and perhaps other echinoderms, as it comes from the ovary is surrounded by a hyaline, radially striated layer which swells in sea water so that the striations disappear, and it becomes invisible. KRTÉ calls this the egg jelly, but it had received many names previously, i. e. chorion, zona pellucida, jelly, mucus etc. It seems to have the physical properties and solubilities of mucus. There are a number of mucins, and the one derived from frog's egg jelly differs chemically from any known mucin from mammals. We will use the word mucus (without attaching any chemical significance to it) rather than jelly, since the perivitelline substance seems also to be a jelly.

The egg of *Toxopneustes variegatus*, from which the mucus has been removed, does not show a fertilization membrane, although it segments after fertilization³. In an electric current the mucus and egg move toward the anode and the perivitelline jelly toward the cathode. This observation has been confirmed last summer, and shows that the mucus is charged negatively and the perivitelline colloid positively. Since oppositely charged colloids precipitate one another, I advanced the hypothesis in 1911 that the fertilization membrane is a precipitation at the contact of the mucus with the perivitelline colloid, and for that reason removal of the mucus prevents the formation of the membrane⁴.

The mucus may be removed from *Toxopneustes* eggs by washing for an hour or so in large quantities of the sea water near Key West, Florida (which is hyperalkaline as compared with Woods Hole sea water). The mucus is more difficult to remove from *Arbacia* eggs at Woods Hole, but I have succeeded in doing so by each of the following methods.

1. Rapid centrifuging in haematokrit tubes two or three times, the whole operation taking but a few minutes. The eggs are suspended in fresh sea water each time and the centrifuge started with a sudden jerk.

¹ McCLENDON, Amer. Jour. Physiol., XXVII, p. 253, 1910.

² For apparatus see McCLENDON, On the Electric Charge of Protoplasm and Other Substances in Living Cells, this heft, fig. A.

³ McCLENDON, Amer. Jour. Physiol., XXVII, p. 240, 1910.

⁴ McCLENDON, Science, XXXIII, p. 387, 1912.

2. Washing not more than twenty-four hours in large quantities of sea water in rotating flask filled and closed so as to exclude air bubbles, since the latter injure the eggs.

3. Washing less than 2 ccm of eggs in 10 litres of sea water agitated with an automatic glass stirring rod not more than twenty-four hours. Controls showed that mere standing in sea water during the time required for these operations did not remove the mucus or prevent the appearance of a fertilization membrane.

The presence or absence of the mucus was determined in each case by at least two of the following methods:

First, observing the eggs in a single layer in Chinese or India ink suspension in sea water.

Second, staining the mucus with neutral red, mythylene blue or toluidin blue. Continuous observation is necessary since the dye first stains the outer surface of the mucus, but finally stains all of it and causes it to contract so close to the egg as to be indistinguishable from the egg.

Third, the massing of spermatozoa which become entangled in the mucus¹.

In every case in which the mucus was completely removed before fertilization, no fertilization membrane could be observed after sperm were added, nevertheless the eggs segmented. The same results were obtained by ELDER² on a California sea urchin, so we now have uniform results on sea urchins in Florida, Woods Hole and California.

It might be objected that the eggs were injured by removal of the mucus and this injury prevented the formation of fertilization membranes. Such an objection cannot be answered directly, but ELDER and I used several methods for removing the mucus and the eggs were not injured so as to prevent segmentation.

If my hypothesis of the formation of the fertilization membrane is correct, neither the fertilization membrane nor the perivitelline substance should be found on the surface of the unfertilized egg.

I failed to find a membrane on the surface of the unfertilized egg that resisted puncture by a glass needle so much as the fertilization

¹ ELDER in the paper cited below claims that the mucus attracts the spermatozoa but apparently it merely mechanically entangles them as stated by F. LILLIE. I removed the mucus from *Arbacia* eggs and washed them repeatedly for ten days. During all this time they gave out a sperm attracting substance which may be merely CO₂, since CO₂ attracts them.

² Arch. f. Entw.-Mech. XXXV, p. 195, 1913.

membrane did. I have frequently observed a spermatozoan wriggle through the mucus until its head touched the fertilization membrane, when its progress was stopped, although its tail continued to vibrate as vigorously as before. Previous observers have found that unfertilized eggs are more easily broken up than eggs with membranes by shaking with powdered glass or sand.

These observations show that the characteristically tough fertilization membrane does not surround the unfertilized egg.

The process of "membrane formation" was observed in eggs of *Arbacia* and *Asterias* with ZEISS 2 mm, homogen. imm. apochromat. obj. and compens. oc. 12 and lowers powers by daylight; also with a high power dry lens with the ultramicroscope. It was found that certain appearances might be due to the curvature of the surface of the egg. An air bubble the same size as the egg appears to be bounded by a sharp circular line, but both outside and inside this circle are a series of concentric circular lines which grow fainter in proportion to their distance from the sharp line. Let us call them pseudo-lines: The surface of the unfertilized egg has a border zone free from granules and bounded externally by a sharp line. Outside this sharp line appears a series of pseudo-lines the same distance apart as the pseudo-lines around the air bubble. The greatest number I have seen by direct daylight is three, but with the ultramicroscope five are very distinct. The fact that they are variable in number strengthens the view that they are false visual impressions. But why do we not see pseudo-lines inside the sharp line? The answer seems clear. The line of separation of the hyaline border zone and granular interior is in the position that should be occupied by the first pseudo-line and is probably intensified by it, and the other pseudo-lines are obscured by the granular cytoplasm.

In observing fertilized eggs by all the above methods the fertilization membrane at some foci looks like the surface of the air bubble described above, but at a certain focus (which I think is the true focus, the others being out of focus) the sharp line appears double, the two sharp lines apparently forming the inner and outer boundaries of the membrane. I have repeatedly observed eggs just after fertilization when the membrane was separated only over a small part of the surface, and attempted to trace the two sharp lines of the membrane into their relation with the egg surface over which no membrane had yet appeared. One might in this way hope to see whether the membrane arises from the egg cortex or from the mucus, or from both. The pseudo-lines seen with high powers and the obvious difficulties with low powers

prevent a positive answer to this question, but it seems clear that the whole granule free cortex of the egg is not lifted off to form the fertilization membrane, for immediately after the fertilization membrane appears the granule-free cortex is as thick as before. The granule-free zone appears to thicken later to form the hyaline plasma layer.

J. LOEB supposes the perivitelline colloids to be set free at the surface of the egg after fertilization, i. e. not to form the most superficial layer of the egg before fertilization. Such a view seems necessary if my hypothesis is correct, but such a process has not been demonstrated to every one's satisfaction, and my hypothesis remains a hypothesis.

Summary.

If the mucus layer (jelly, chorion, zona pellucida) is removed from the sea urchin's egg before fertilization, no fertilization membrane appears, although the egg segments. No membrane of the toughness that characterizes the fully developed fertilization membrane exists on the surface of the unfertilized egg.

Zusammenfassung.

Wenn die Schleimschicht (Gallerte, Chorion, Zona pellucida) vor der Befruchtung von dem Seeigelei entfernt wird, zeigt sich keine Befruchtungsmembran, obgleich sich das Ei teilt. Es besteht keine Membran von der Zähigkeit, die die voll entwickelte Befruchtungsmembran charakterisiert, an der Oberfläche des nicht-befruchteten Eies.

1220
23

the Parallelism between Increase in Permeability and abnormal Development of Fish Eggs

by

J. F. McClendon

With 4 figures in text

Abdruck aus: »Internat. Zeitschrift für physik.-chem. Biologie.« I. Band, 1. u. 2. Heft

Leipzig und Berlin

Wilhelm Engelmann

1914

On the Parallelism between Increase in Permeability and abnormal Development of Fish Eggs.

By

J. F. McClendon.

(Contribution from the U.S. Bureau of Fisheries, Woods Hole, Mass., and the
Anatomical Department of Cornell University Medical College, New York City,
Prof. C. R. Stockard, Direktor.)

With 4 figures in text.

(Eingegangen am 22. Januar 1914.)

The morphology of abnormal embryos of the marine teleost *Fundulus heteroclitus*, has been studied by STOCKARD¹. My work has been merely an attempt to find the cause of the abnormalities in

eggs, and several preliminary papers have already been published².

The present paper deals mainly with *Fundulus*, the conclusions are supported by work on the eggs of vertebrates and other telebrates.

The abnormal development of the eggs of *Fundulus* may produce an indefinite number of different types. Some of the most striking abnormalities of the embryos seen in the living embryos approach the following

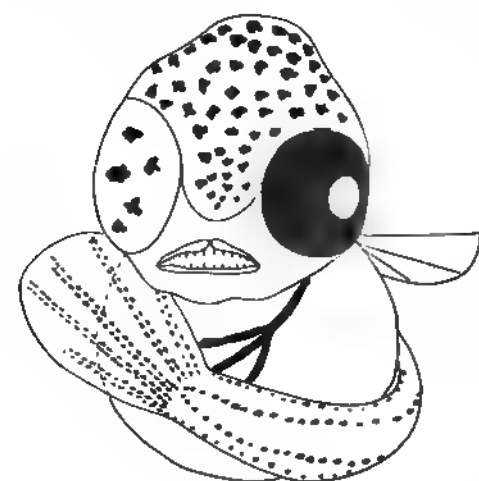


Fig. 1.

types: one eye may be absent (monophthalmia asymmetrica, etc.). The two eyes may be represented by one median eye (cyclopia).

¹ STOCKARD, Jour. Exper. Zool., III, p. 99, 1906, and IV, p. 165, 1907; VI, p. 285, 1909; Amer. Jour. Anat., X, p. 369, 1910.

² McCLENDON, Soc. Exp. Biol. and Med., VII, p. 38, 1910; Amer. Physiol., XXIX, p. 289, 1912; ibid. XXXI, p. 131, 1912.

fig. 2), various cavities, such as the segmentation cavity, pericardium and ear vesicles may be distended (figs. 2 and 3). The heart may be rudimentary (fig. 3). The blood vessels may be rudimentary or apparently absent (fig. 3). The circulation of the blood may be absent. The heart may not beat. There are other abnormalities which I have omitted, but a large percentage of abnormal *Fundulus* embryos show one or more of the above abnormalities or transitions between those types and the normal.



Fig. 2.

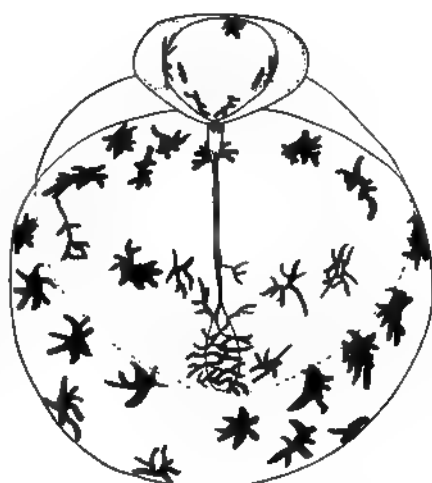


Fig. 3.

Last season I produced all of the above types of abnormalities in *Fundulus* embryos by treating them in early segmentation stages with pure solutions of any one of the following substances: Nicotine or the chlorides or nitrates of sodium, potassium, lithium, calcium or magnesium. Sea water solutions of these substances produced the same results. I thought this similarity of action of such widely different substances might be due to their action in increasing the permeability of the eggs to an abnormal degree.

It was found that the fertilized eggs of the smelt or of *Fundulus heteroclitus*, in distilled water, do not give up salts to the water. This is also true of these eggs when placed in salt solutions in which the kations are balanced. Thus, in an artificial sea water made of the nitrates of sodium, potassium, magnesium and calcium, no chlorides diffuse out of the eggs.

When the eggs were placed in pure solutions of nicotine or of salts of sodium, potassium, calcium, or magnesium, salts diffused out of the eggs and were detected. The detection of some salts or ions was attended with difficulties, as given in the section on technique, and it is obvious that if sodium chloride, for instance, diffused out of the eggs into a pure solution of sodium chloride, the most delicate quantitative methods might not suffice for its detection.

Absolutely trustworthy results were obtained on the diffusion of chlorine and magnesium ions, especially in the case of *Fundulus* eggs, the experiments with smelt eggs being less numerous. The results on the diffusion of sodium, potassium and calcium are based on isolated experiments (i. e., not repeated). The following table demonstrates the increase in permeability of *Fundulus* eggs.

Pure solution in which eggs were placed	Ions which diffused out of eggs and were detected
Nicotine	Cl, Mg, Ca, Na, K
NaCl	Mg, Ca, K
NaNO ₃	Cl, Mg, Ca, K
KCl	Mg, Ca, Na
KNO ₃	Cl, Mg, Ca, Na
Mg(NO ₃) ₂	Cl, Na, K
Ca(NO ₃) ₂	Cl, Na, K

Though a number of different concentrations of these solutions were found to be effective, they will not all be given, as this phase of the subject was not completely worked out. Let it suffice for the present to say that the following concentrations in distilled water were effective on *Fundulus* eggs:

KCl or KNO ₃2 molecular
NaCl or NaNO ₃3 "
Mg(NO ₃) ₂3 "
Ca(NO ₃) ₂15 "
Nicotine002 "

Each of these solutions, when applied in early segmentation stages, caused all of the types of monstrosities listed above. All concentrations of these solutions which caused these monstrosities, increased the permeability of the eggs. Furthermore, the permeability of the eggs was slightly increased by certain solutions too weak or acting for too short a time to produce monstrosities. Therefore, the increase in permeability is the cause or is associated with the cause of the monstrosities and is not merely an effect, i. e., one of the characteristics of monstrous embryos.

The increase in permeability probably occurs in the hypothetical "plasma membrane", as the shell or chorion is permeable to salts and water, though not freely permeable to sugar.

Possibly the permeability of certain cells or parts of the egg is increased allowing abnormal diffusion of some substance such as oxygen, CO₂, sugar or amino acid. Or the free diffusion of ions may abolish the electric polarization shown by HYDE¹ and increase the surface tension.

Material and Methods.

There are many sources of error in the electrolytic, plasmolytic and microchemical methods for determining permeability and I decided to use chemical methods. Failing to find chemical methods that could be applied to the eggs of invertebrates I chose the eggs of the smelt and of *Fundulus heteroclitus*.

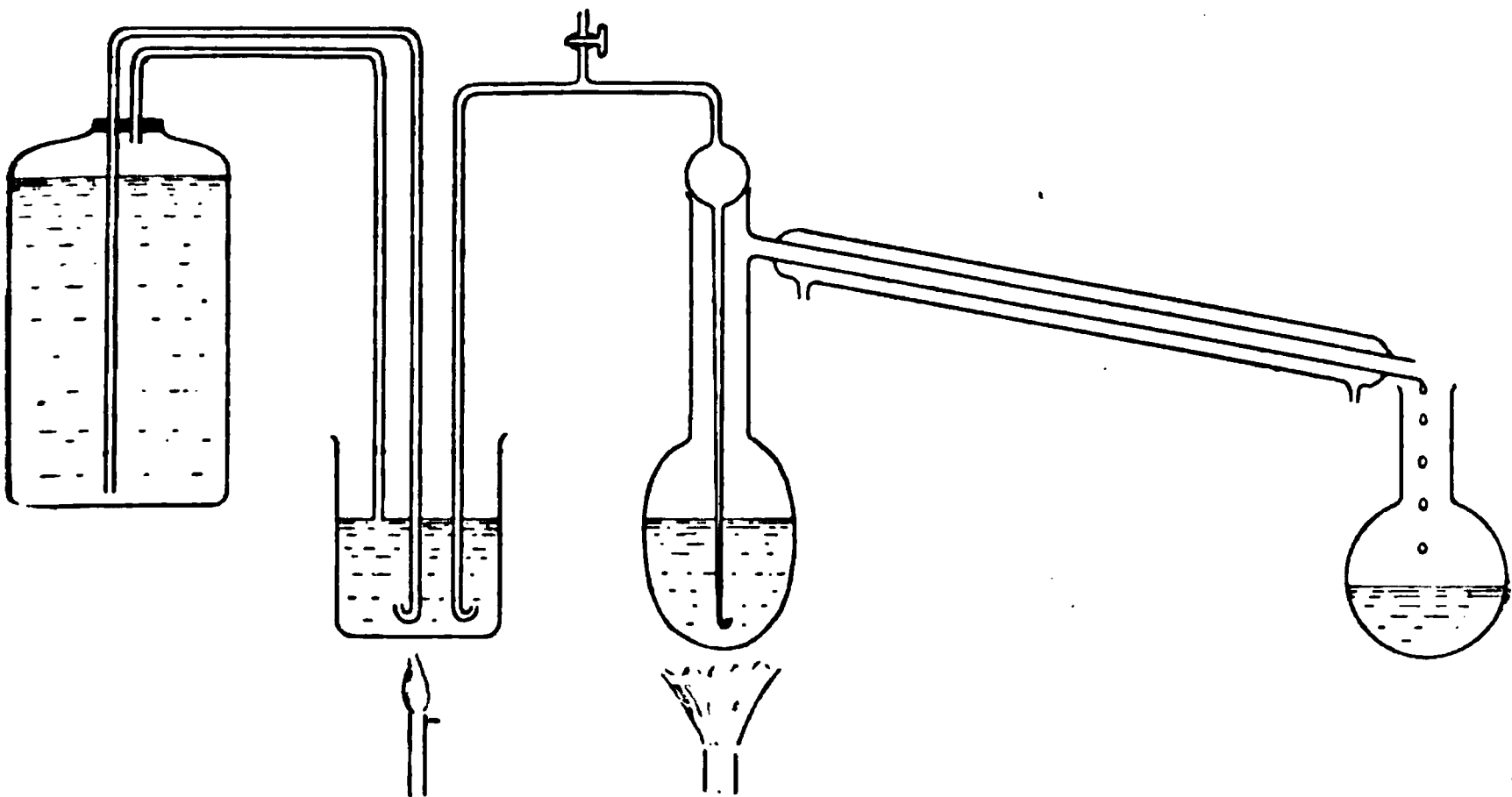


Fig. 4. Quartz still.

Clear spring water, distilled in a tin lined still, is placed in the stock bottle to the left, and from this it syphons over into the beaker. A small flame under the beaker boils out CO₂ and some other gaseous impurities. The water syphons over from the beaker into the kjeldahl flask. A bulb is blown on this syphon to close the mouth of the flask. A hole is blown in the neck of the kjeldahl flask and reamed out while soft to admit the end of the quartz condensing tube. Only water that condenses in the quartz tube can drip into the quartz flask at the right.

The eggs of the smelt will develop normally in redistilled water and in sea water diluted to about 4 times its original volume.

The eggs of *Fundulus* will develop in redistilled water, sea water or even in sea water concentrated to $\frac{1}{2}$ its original volume.

¹ Am. Jour. Physiol., XII, p. 241, 1904.

The eggs of both species appear to be impermeable to water and to salts. When placed in redistilled water salts do not diffuse out of them unless the permeability has been artificially increased. It might be supposed that the salts were held in chemical combination or in indiffusible form in the eggs, and this is probably true of some phosphates, for instance, but omitting phosphates and carbonates of alkaline earth metals, the egg of *Fundulus heteroclitus* contains as high a percent of soluble ash (3,18) as the local sea water (2,84—3,29). Furthermore HÖBER has brought forward much evidence to show that at least a part of the salts in living cells is in a freely diffusible form and therefore their retention by the cell may indicate impermeability.

Ordinary distilled water was found to be slightly toxic to the eggs, and therefore all of the water used in the experiments was redistilled in quartz in the still shown in fig. 4.

The general method is best explained by describing one experiment. Fifty cc. of smelt eggs taken as soon as possible after fertilization were washed in repeated changes of redistilled water and the time when silver nitrate no longer gave an opalescence to the washings noted. The washing was then continued about 2 hours longer. The mass of eggs was then divided into 2 equal parts (a and b) and treated separately as follows:

a (control)	b
25 cc. eggs placed in 500 cc redistilled water in quartz vessels for 30 minutes.	25 cc. eggs placed in 500 cc. redistilled water plus .6 gram NaNO_3 in quartz vessels for 30 minutes.
400 cc. water poured from eggs and evaporated in quartz to 50 cc. and .6 gram NaNO_3 added.	400 cc. solution poured from eggs and evaporated in quartz to 50 cc.
1 cc. N/10 AgNO_3 added, solution remains clear.	1 cc N/10 AgNO_3 added, forming dense white cloud, due to chlorides that had diffused out of eggs.
Eggs kept in redistilled water and development watched.	Eggs kept in the remaining 100 cc NaNO_3 solution and at intervals of time some are transferred to redistilled water and development watched.

A separate experiment was made for the detection of each ion.

The quantities of *Fundulus* eggs were necessarily less (usually about 10 cc). This disadvantage was partly compensated by exposing them for a longer time, but owing to the disadvantages of exposing

them for a very much longer time, the operation was done on a smaller scale, each solution being concentrated in a platinum crucible to 2 cc. or less before analysis.

In the event that some eggs died or bacteria attacked their surfaces or membranes, the experiment was thrown out. Even if bacterial action was too slight to give opalescence to the solution, haptogen membranes or coagula were noted on boiling down the solution and it was thrown out. The presence of a trace of reducing substance was not considered sufficient to vitiate the results, since such titrations as with silver nitrate were done quickly, and, even though allowed to stand a reduction would be detected by a change of color. Furthermore, all precipitates were examined under the microscope and the form of the crystals studied.

Most reliance was placed on precipitation methods, but these will not be discussed here as it is hoped to work them out to a fine point quantitatively in the future.

In addition to these precipitation methods a prism spectroscope was used. A grating spectroscope was found too dim for such small quantities of substance. In observing the spectrum of magnesium, high potential electric sparks were allowed to pass from the surface of the solution to a platinum electrode. In looking for other elements a platinum loop with or without a bit of asbestos wool was dipped in the concentrate solution and held in the flame observed with the spectroscope. The asbestos wool, after being thoroughly cleaned could be used only once. The platinum loop was dipped into pure hydrochloric acid and heated to a white heat and this process repeated about seven times, or until it gave no sodium or other lines to the flame. Traces of sodium were always present in the air. Although the Bunsen burner was washed in many changes of distilled water and dried, and the gas was filtered through packed cotton wool, minute flashes of sodium light appeared in the flame and were caused by sodium in the air drawn into the Bunsen burner.

The chemical reagents were the purest that could be obtained and were tested immediately before being used. Minute traces of impurities could not lead to erroneous conclusions owing to the character of the control, but they might, and in some cases did lead to the negative results, and lead me to discontinue experiments begun in 1911.

Conclusions.

Distilled or sea water solutions of nicotine or the salts of Li, Na, K, Ca or Mg all produce the same abnormalities in the development of certain fish eggs placed in them in early segmentation stages.

These substances increase the permeability of the eggs to salts or their ions (the eggs being impermeable to salts in pure water or "balanced" salt solutions).

Since the increase in permeability occurs before the abnormalities appear it is probable that the abnormal permeability is the cause of the structural abnormalities.

Zusammenfassung.

Werden bestimmte Fischeier in frühen Teilungsstadien in Lösungen von Nikotin oder den Salzen von Li, Na, K, Ca oder Mg in destilliertes oder Seewasser eingesetzt, so werden in allen Fällen dieselben Mißbildungen in ihrer Entwicklung hervorgebracht.

Während die Eier in reinem Wasser oder in ausgeglichenen (balanced) Salzlösungen für Salze undurchlässig sind, wird eine Durchlässigkeit der Eier für Salze oder deren Ionen durch obige Stoffe herbeigeführt.

Da diese Permeabilitätsänderung eintritt, bevor die Mißbildungen erfolgen, so ist jene abnorme Durchlässigkeit wahrscheinlich die Ursache der Mißbildungen.

THE ACTION OF ANESTHETICS IN PREVENTING INCREASE OF CELL PERMEABILITY¹

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Received for publication May 5, 1915

Höber and Lillie have attempted to associate anesthesia with permeability. In a lecture at Woods Hole in 1911, I attempted to express the relation as follows: "If stimulation consists in increase in permeability, we should expect anesthetics to prevent this change."²

Most of the attempts at correlating anesthesia and permeability have been indirect. Thus Höber³ found that anesthetics prevent the action of salts in producing a demarcation current in muscle. Lillie⁴ observed that anesthetics prevent the action of salt solutions in causing artificial parthenogenesis and cytolysis of Echinoderm eggs. He also observed that anesthetics prevent the outward diffusion of cell pigment produced by salts, and thinks this a direct proof that anesthetics prevent increase in permeability. Arrhenius and Bubonovic⁵ observed that anesthetics retard the hemolytic action of hypotonic solutions. Höber attributes a similar observation to Traube, but I have not as yet found the reference.

It is clear, therefore, that anesthetics in the anesthetizing concentration, tend to prevent the outward diffusion of pigment from pigmented cells. It seemed to me of interest to determine whether non-pigmented substances are affected in this way, especially the salts contained in the cells. In 1911, I had observed that the *Fundulus* egg is impermeable to salts, but found that Mg came out of the eggs when placed in a slightly toxic solution of NaCl.⁶

¹ This research assisted by a grant from the Research Fund of the Graduate School.

² McClendon: Biol. Bull., 1912, xxii, 139.

³ Höber: Pflüger's Arch., 1907, cxx, 492, 501, 508.

⁴ Lillie: This Journal, 1912, xxix, 373, 1912, xxx, 1; 1913, xxxi, 255; Journ. Exper. Zool., 1914, xvi, 591.

⁵ Arrhenius and Bubonovic: Meddel. K. Ventensk. Akad. Nobel institut, 1913, ii, no. 32.

⁶ McClendon: This Journal, 1912, xxix, 296.

It was later found that various toxic solutions increase the permeability of the egg to salts.⁷ The addition of anesthetics to these salt solutions tends to prevent this increase in permeability.⁸

In making these last experiments, *Fundulus* could not be obtained and the much more delicate eggs of the pike were used. It was found that practically no salts came out of these eggs when they were placed in distilled water. Since the egg contains salts, and it does not swell or burst when placed in distilled water, it must be impermeable to water as well as to salts. When placed in slightly toxic solutions of nitrates or anesthetics the chlorides diffuse out of the egg very rapidly. But when a toxic salt solution ($\frac{1}{10}$ molecular NaNO_3) contains an anesthetic in the proper concentration for anesthesia, the chlorides diffuse out of the egg at a slower rate than in the pure salt solution. Thus anesthetics have two effects on these eggs, at a certain concentration they produce anesthesia and at a greater concentration they are toxic. A 3 per cent solution of alcohol or a 0.5 per cent solution of ether produces anesthesia (retards development) whereas a 6 per cent solution of alcohol, or a 2 per cent solution of ether is slightly toxic. Twice as much chloride diffuses out of eggs placed in pure NaNO_3 solution as in the same solution containing 3 per cent alcohol. One and a half times as much chloride diffuses out of the egg in the pure NaNO_3 solution as in the same solution containing 0.5 per cent ether.

These experiments were extended this year, and the chlorides determined more accurately with a Richards' nephelometer.

MATERIAL

The eggs of the pike (*Esox*) were used. These eggs are very delicate, and are especially susceptible to changes in temperature, lack of oxygen and to the presence of various substances in the medium. The eggs were shipped from the breeding grounds in northern Minnesota to the State Fish Hatchery in Saint Paul by being spread out in thin layers on stretched muslin in refrigerator boxes. From this point they were brought to the laboratory in a refrigerator box, and those in good condition selected one by one and placed in water that was distilled, boiled and later redistilled in an automatic quartz still.⁷ No other water was used in the experiments.

⁷ McClendon: Internat. Zeitschr. f. Physik.-chem. Biol., 1914, i, 28.

⁸ McClendon: Sci., 1904, xxxviii, 280.

METHODS

The eggs are washed in the redistilled water until the salts practically cease coming out of them, and measured in a series of graduated cylinders of the same bore. That the chlorides have all been washed out of the transparent egg shell follows from the following facts: The last wash waters contain so little chlorides that they cannot be detected with the nephelometer unless the washings are boiled down to a small volume. Tap water usually contains 100 to 10,000 times as much chlorides. The eggs are made permeable to chlorides by $\frac{1}{10}$ molecular NaNO_3 solution, and while they remain in this, twice as much chloride diffuses out of the eggs in six hours as in three hours. If these chlorides were held in the thin shell we would expect more to come out in the first three hours than in the second three hours. Since the shell is at all times easily permeable to salts, the source of the chlorides is not the fluid between the egg and the shell, and the small amount of shell substance is probably not the source of such large amounts of chlorides as were obtained from the eggs. I found that when the *Fundulus* egg is made permeable to chlorides the other salts diffuse out also, and when the frog's egg is made permeable to Cl that SO_4 , CO_3 , Na, K, Li, Mg and Ca diffused out also. This is probably true of the pike egg also, and enough salts could probably be obtained by diffusion to equal the entire weight of the egg shell, which is protein in composition. The most reasonable hypothesis is that the plasma membrane is practically impermeable to salts (and to water) and that it is made permeable by toxic solutions. The permeable egg may live and develop for several days, showing that the entire store of salts is not necessary for its immediate needs.

EXPERIMENTS

The same volume of eggs was placed in each of a series of Stender dishes, with 30 cc. of the solution to be tried. At the end of six hours, 25 cc. of this solution was removed with a pipette having a trap to prevent contamination with saliva. The sample was transferred to the nephelometer tube, and five drops of a saturated solution of AgNO_3 added and mixed. The two matched test tubes used in the nephelometer were filled up to the zero point of the scale by the 25 cc. + 5 drops.

In case any eggs died during an experiment, this experiment was thrown out. Since the material was available in great abundance,

a number of experiments were performed, of which the following are typical:

(1) 5 cc. of eggs in each dish. Chlorides expressed in arbitrary units. Ethyl alcohol expressed in per cent by volume.

Solution.....	$\frac{N}{10}$ NaNO ₃	+1% alcohol	+2% alc.	+3% alc.	+4% alc.
Chlorides.....	100	60	50	45	60

(2) 15 cc. of eggs in each dish

Solution.....	$\frac{N}{10}$ NaNO ₃	$\frac{N}{10}$ NaNO ₃ +2% alc.	$\frac{N}{10}$ NaNO ₃ +3% alc.
Chlorides.....	100	50	40

The duration of the experiment, six hours, was selected because this was the maximum duration that could be considered safe. Even then, some of the experiments had to be thrown out because one or two eggs died before they were completed. The NaNO₃ has to be pure in order to have the right degree of toxicity. The ethyl alcohol was redistilled over metallic sodium. Although the dishes were covered in all experiments, the experiments with ether were discontinued after it was found that ether has the same effect as alcohol, owing to the fact that some of the ether diffused into the air above the eggs and the concentration of that remaining in the solution was no longer known. It is probable that all anesthetics have the same action.

A word of caution to any one who wishes to make similar experiments on pike eggs: Some of my early experiments were irregular in results. I believe the reason for this is that the egg is made more permeable by increase in temperature,⁹ and that different eggs are not affected by the same temperature. I did not determine the maximum temperature at which it is safe to work, but a temperature of about 8° is safe and is easily maintained in a refrigerator. Fundulus eggs may be used at room temperature in Woods Hole, and are preferable in every way. It is clear that 2 to 3 per cent by volume of ethyl alcohol partially inhibits the permeability-increasing action of a pure NaNO₃ solution. It is theoretically possible to entirely prevent this increase in permeability if the toxicity of the NaNO₃ solution is low enough. The difficulty in demonstrating this lies in the fact that the toxicity of the sodium salt does not depend on its absolute concentration, but

⁹ Osterhout: Biochem. Zeitschr., 1914, lxxvii, 272.

on the ratio of sodium to calcium. With a very mildly toxic solution of NaNO_3 the Ca diffusing out of the egg at the first increase of permeability lowers the toxicity and hence the permeability to such an extent that no permeability increase can be measured by means of the nephelometer, and electric conductivity experiments with their large and numerous sources of error would have to be substituted.

That 2-3 per cent ethyl alcohol is really the anesthetic concentration, follows from the fact that it retards the development of these eggs. The same concentration may not be correct for every species or every tissue. In general, it seems that nerve tissue requires a less concentration of an anesthetic for anesthesia than other tissues. But it is hardly justifiable to assume that this affect of anesthetics on permeability is peculiar to egg tissue. Pike embryos were found to behave the same as eggs up to the time of the development of kidney function, when the excretion of salts interferes with the method used to measure permeability. It is probable that anesthetics retard the increase in permeability of any cell by any "stimulus."

DISCUSSION

In 1910 I observed that chloroform, when added to the sea water, reduces the electric conductivity of sea urchin eggs. This experiment was not repeated, owing to lack of time and the large number of sources of error that must be guarded against in order to be sure that decreased conductivity indicates decreased permeability. Osterhout,¹⁰ by finding a tougher material, was able to show that anesthetics decrease the permeability, at least of certain plant cells. Joel¹¹ found that anesthetics decrease the permeability of erythrocytes. The question arises whether anesthetics prevent increase in permeability by decreasing permeability. If a cell is absolutely impermeable its permeability cannot be decreased. The *Fundulus* egg is so nearly impermeable to salts that it would be extremely difficult to measure a decrease in permeability. The pike egg may be more permeable to salts but it would be no easier to measure a decrease in permeability, owing to the delicate nature of the egg and danger of non-uniformity of material. It seems probable, however, that the anesthetic and toxic substances act on the same constituent of the cell surface or plasma membrane. If the cell surface is composed of a mosaic of different constituents,

¹⁰ Osterhout: Sci., 1913, 111.

¹¹ Joel: Pflüger's Arch., 1914.

and one constituent is permeable, the cell is permeable. But if one constituent is impermeable the whole cell is not necessarily impermeable, since diffusion can take place through the other constituent. If the NaNO_3 makes the cell permeable by acting on a protein it is difficult to see how the anesthetic could antagonize this effect by acting on a lipoid.

Stimulation and anesthesia seem to be antagonistic states. I have shown that the permeability of striated muscle is increased on stimulation¹² and that the permeability of the eggs of the sea urchin and the frog increases when they pass from the state of repose into that of activity.¹³ The question arises whether this is true of other cells, such as those of glands. In order to decide this we must discuss the psycho-galvanic reflex.

If a constant electric current is sent through the body from non-polarizable electrodes and the person is given a nervous shock, as by sticking a pin in him unexpectedly, the strength of the current is momentarily increased. Leva¹⁴ showed that the degree of this change is in direct ratio with the number of sweat glands per unit area of the skin, and therefore concluded that the sweat glands produce this phenomenon. Gildemeister¹⁵ was able, by improvements in the electric conductivity method, to show that this is due to the increased permeability, presumably of these glands. Apparently the gland cells are stimulated by the sympathetic nerves and their permeability is increased.

It seems, therefore, that the increased permeability on stimulation is a general phenomenon, and we might expect the action of anesthetics in preventing this increase to be general, also.

Warburg¹⁶ has shown that anesthetics retard the oxidation of oxalic acid by blood charcoal to about the same degree as they decrease the respiration of nucleated erythrocytes. I have repeated and confirmed these experiments. Tashiro and Adams¹⁷ have shown that the nerve fiber gives out less CO_2 when it is anesthetized. Although it was shown by Warburg¹⁸ that the respiration of sea urchin eggs is only slightly

¹² McClendon: This Journal, 1912, xxix, 302.

¹³ McClendon: This Journal, 1910, xxvii, 240, and *ibid*, in press.

¹⁴ Leva: Münch. Med. Wochenschr., 1913, 2386.

¹⁵ Gildemeister: Münch. Med. Wochenschr., 1913, 2289; see also Schwartz: Zentbl. f. Physiol., 1913, xxvii, 734.

¹⁶ Warburg: Pflüger's arch., 1914, clv, 147.

¹⁷ Tashiro and Adams: Int. Zeitschr. f. Physik.-chem. Biol., 1914, i, 451.

¹⁸ Warburg: Zeitschr. f. Physiol. Chem., 1910, lxvi, 306.

reduced during anesthesia, it seems generally true that anesthetics may antagonize oxidations by cells, oxidases and some inorganic katalyzers. The question whether permeability has any relation to the oxidative processes cannot be finally settled until we know more about the mechanism of the latter. At present we can, at most, make the generalization that in the presence of O_2 cell respiration varies more or less with cell permeability so long¹⁹ as the cell is alive. The only observation that I know of that might appear to extend this rule to a dead cell is that of Warburg.²⁰ He found that the oxidation in the young erythrocytes of the goose is increased by freezing and thawing provided they are closely packed. Freezing and thawing causes hemolysis and increases permeability, and the hemolyzed cells might be considered dead.

SUMMARY

Anesthetics in the concentration that retards development (2-3 per cent alcohol or 0.5 per cent ether) tends to inhibit the permeability-increasing action of a $\frac{1}{10}$ molecular solution of $NaNO_3$ on the eggs and embryos of the pike (*Esox*).

¹⁹ McClendon and Mitchell: Journ. Biol. Chem., 1912, x, 459.

²⁰ Warburg: Zeitschr. f. Physiol. Chem., 1911, 419.

DIFFERENCES IN THE DIGESTION IN ADULTS AND INFANTS

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The differences in the digestive process in adults and infants have been the subject of so many contributions that it would be impossible to review the literature in a paper of this length; but some recent work is of especial interest in shedding new light on this subject. We are still very ignorant of the chemical nature of the digestive enzymes, but are learning more of the conditions under which they act.

Sorensen¹ has made careful studies of the relation between the acidity or alkalinity of the solution and the rapidity of the action of different enzymes. The acidity is measured by the concentration of the hydrogen ions. The optimum concentration of hydrogen ions for peptic digestion was found to be about 0.04 normal, and the rate decreased if the acidity was increased or decreased. At about 0.0001 normal it was about a half to a fifth as rapid, and at about 0.2 normal it was about 0.9 to 0.5 as rapid, depending on the duration of the experiment and the method of analysis.

It is well known that trypsin acts better in an alkaline medium, and Michaelis and Davidsohn² have shown that its action decreases as the alkalinity decreases, and still more if the solution becomes acid, so that at a hydrogen ion concentration of 0.0001, it ceases altogether. Rona and Arnheim³ have found the same thing true of erepsin.

It occurred to me that the rate of digestion in the stomach and intestine might be studied by continuous observation of the hydrogen ion concentration. For

1. Sorensen, S. P. L.: Biochem. Ztschr., 1909, xxi, 131.

2. Michaelis and Davidsohn: Biochem. Ztschr., 1911, xxxviii, 280.

3. Rona and Arnheim: Biochem. Ztschr., 1913, lvii, 84.

Received, A. 1112
July 3, 1915

this purpose new apparatus was perfected,⁴ one method being the construction of a hydrogen electrode which could be lowered into the stomach. In this way curves were plotted of the rise in acidity in the stomach.⁵ The other method consisted in the use of an Einhorn duodenal tube kept in the stomach.

Although all the determinations were made with the hydrogen electrode, the indicator method of determining the reaction was tested. When the sample was already colored, indicator papers were found to work better than the solutions. Ordinary indicator papers are not always sensitive enough. It is better to soak Schleicher and Schüll's ash-free filter paper No. 589 in a solution of the dye and dry it. In case of a natural indicator, if it will dissolve in absolute alcohol, this solution is used to color the paper in order that no salts may be transferred to the paper. The edge of the paper is touched to the solution, and as the solution rises in the pores of the paper, the coloring matters are precipitated in the pores; but the acid or alkali rises higher and gives the characteristic reaction with the indicator above the place where the color of the sample is precipitated. Pure water rises still higher; therefore the upper limit of the moist area should not be taken to indicate the reaction.

The hydrogen ion concentrations at which some indicators change the contents of the digestive tract are as follows: methyl violet and tropeolin 00, 0.01; dimethylamidobenzol, 0.005; congo, methyl orange and alizarin, 0.0001; litmus and p-nitrophenol, 0.00001; neutral red, 0.000005; rosolic acid, 0.0000005; phenolphthalein, 0.00000005.

It was found that the heavier the meal and the more protein it contained, the more slowly the acidity rose. After the average meal, however, the acidity rises rapidly during the first hour, more slowly during the second hour and remains about stationary during the third, fourth and fifth hours. During the first hour very little peptic digestion can take place, and the most

4. McClendon, J. F.: New Hydrogen Electrodes and Rapid Methods of Determining Hydrogen Ion Concentrations, *Am. Jour. Physiol.*, July, 1915; A Direct Reading Potentiometer for Measuring Hydrogen Ion Concentrations, *ibid.*

5. McClendon, J. F.: Acidity Curves in the Stomachs and Duodenums of Adults and Infants, as Plotted with the Aid of Improved Methods of Measuring Hydrogen Ion Concentrations, *Am. Jour. Physiol.*, July, 1915.

rapid digestion is delayed until from two to three hours after finishing the meal.

The acidity curve is thus an index of the efficiency of the stomach to take care of a meal, and a whole hour is saved by the ability of the stomach to raise the acidity an hour sooner. The acidity rises to its maximum an hour sooner in some stomachs than in others, and these stomachs are emptied through the pylorus sooner than the others, and hence made ready for another meal.

This brings us to the question of the infant's stomach, and it was investigated at the request of Dr. Sedgwick in the same way so far as was practicable. In this work Dr. Rood Taylor assisted, and collected all samples. The acidity of the infant's stomach (of the first month) remains nearly stationary during the first hour, after which it rises steadily until the next meal, and if this is delayed four hours it may become as acid as the adult stomach. The question arises as to what this has to do with the digestion. The milk does not wait to be digested before passing out of the stomach, and when the acidity is high enough for protein digestion, there is little milk in it to be digested.

A study of the duodenal contents offers a suggestion in explanation of this anomaly. The duodenal contents are more acid than the full stomach, the hydrogen ion concentration being about 0.0008, despite the presence of bile and pancreatic juice. At this acidity, considerable peptic digestion could take place, according to the careful studies of Sorensen. Pepsin was looked for and found to be always present in the infant's duodenum. In determining this, 0.1 c.c. of duodenal contents was mixed with 2 c.c. of a dilute solution of edestin kept slightly acid with a phosphate mixture, and incubated for two hours at 37 C. (98.6 F.). At the end of that time the undigested edestin was precipitated with a few drops of a saturated solution of sodium chlorid. A precipitate was observed only in the controls, however, owing to the rapid peptic digestion.

Trypsin was likewise determined, by the digestion of a dilute alkaline solution of casein for twenty-four hours in an incubator. It seemed to be much less abundant than the pepsin, however, from the fact that the casein was not always completely digested in

twenty-four hours; but some undigested casein could be precipitated by neutralization of the solution with acetic acid.

The reaction of the duodenal contents of the infant is such as would allow peptic and tryptic digestion to occur simultaneously, and the question arises, whether or not this reaction is maintained in the ileum. Owing to our inability to obtain intestinal juice, comparative data must be used in this discussion. The feces of the infant are acid,⁶ and it is simplest to assume that the intestine is acid all the way down rather than that it is first acid, then alkaline and then acid again. Furthermore, it is chiefly the pancreatic juice that makes the adult intestinal contents alkaline (Boldyreff). The adult duodenal contents are alkaline, notwithstanding the highly acid fluid coming down from the stomach. I found the hydrogen ion concentration of the adult duodenal contents to be about 0.00000002, and it was not made acid by pouring in a fair proportion of gastric juice. This is about the same alkalinity as found by Auerbach and Pick⁷ in the intestine of dogs, and seems to be characteristic for the adult mammalian intestine. The content of the common bile duct is a fluid like a carbonate solution, whose reaction is not easily changed, and which gives the characteristic reaction to the contents of the small intestine. The same reaction is characteristic of the feces according to Michaelis.⁶ The production of bile in the infant is very small, and is not nearly sufficient to neutralize the gastric juice; consequently it is probable that all of the digestion in the infant takes place in an acid medium.

It is well known that the infant's stomach is especially rich in lipase. In addition to the lipase in mother's milk, the stomach secretes lipase. I found the fat-splitting action of the duodenal contents of the infant to be as great in an acid medium as in an alkaline medium. A saturated solution of tributyrin was used, and the digestion determined by measuring the surface tension with a stalagometer. This test is very delicate, and if any difference existed it should have been detected. The failure to find a difference indicates that the lipase coming from the stomach is so abundant as to make the detection of pancreatic lipase

6. Michaelis: Die Wasserstoffionenkoncentration, Berlin, 1914, p. 112.

7. Auerbach and Pick: Arb. a. d. k. Gsndhtsamte, Berlin, 1912, xlii, 155.

in the mixture difficult. At any rate, the fat-splitting action of the duodenal contents is very rapid in an acid medium, and since there is no starch to be split, there is no reason to suppose that an alkaline reaction is necessary for the infant's digestion.

Since the infant may some day become a man, the question arises as to what becomes of the digestion in the transition process. We observed that the infant's duodenum is not too acid for tryptic digestion, and therefore, when the acidity of the intestine in the growing child decreases so that peptic digestion can no longer take place, it is still equipped with an efficient agent for proteolysis.

The study of the acidity of the stomach during this transition period was begun by Huenekens.⁸ He found that the acidity of the infant's stomach remained low at least five years if it was fed on milk, but on a mixed diet excluding milk and including meat, the acidity of the 17-months-old infant is sufficient for peptic digestion. The proteins of the milk bind and partially neutralize the acid. My results indicate that no stimulus is necessary for the secretion of acid, but the question could not be decided without finding some means of estimating the total quantity of acid secreted, which cannot be done by the titration method unless carbonates and phosphates and proteins are entirely excluded from the stomach.

Since writing this paper I have read that of Rehfuess, Bergeim and Hawk⁹ describing acidity curves by the titration method. Their curves rise more rapidly and then fall. The rise is probably due to the use of the Ewald meal with low acid binding power, and the collection of the sample next to the wall of the stomach. The fall may be due to the regurgitation of duodenal contents. My curves are not complete, and hence show no fall at the end.

SUMMARY

The acidity of the stomach and reaction of the duodenal contents was measured by means of hydrogen electrodes. Indicator papers were calibrated so that clinicians may determine hydrogen ion concentrations approximately.

8. Huenekens: *Ztschr. f. Kinderh.*, 1914, xi, 297.

9. Rehfuess, M. E.; Bergeim, Olaf, and Hawk, P. B.: *Gastro-Intestinal Studies, II, The Fractional Study of Gastric Digestion*, *THE JOURNAL A. M. A.*, Sept. 12, 1914, p. 909.

After a normal meal, the acidity of the adult stomach reaches its maximum in from two to three hours, the rise in acidity being more rapid the lighter the meal.

The height to which the acidity rises varies with the individual, the highest observed in a normal individual being 0.1 normal (about 0.4 per cent. of pure hydrochloric acid). The hydrogen ion concentration of the duodenal contents is 0.00000002 normal. This is slightly alkaline, since that of pure water is 0.00000011 at 25 C. (77 F.).

The acidity of the infant's stomach rises slowly after the milk begins to leave it, and four hours after nursing may be the same as some normal adult stomachs. That of the gastric juice of the new-born is 0.005.

The acidity of the duodenal contents of the infant is 0.0008, and hence it is probable that both peptic and tryptic digestion take place in the intestine of the infant. Pepsin was always found and was apparently more abundant (active) than the trypsin.

*Reprinted from The Journal of the American Medical Association
July 3, 1915, Vol. LXV, pp. 12-14*

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American Medical Association, 535 N. Dearborn St., Chicago

**FERTILIZATION AND ARTIFICIAL PARTHENOGENESIS
OF THE EGG**

BY DR. J. E. McCLENDON

Reprinted from POPULAR SCIENCE MONTHLY, June 1915

FERTILIZATION AND ARTIFICIAL PARTHENOGENESIS OF THE EGG

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TICHOMIROFF, in 1886, was the first to use the term artificial parthenogenesis, referring to acceleration in the development of the naturally parthenogenetic eggs of the silkworm by methods found effective in hastening development in fertilized eggs of the same species. To-day the term is applied to development of eggs not usually parthenogenetic, although a few such might develop in nature under accidentally abnormal conditions.

The exact extent of development that is to be dignified by this term is a matter of dispute, some claiming it should be possible to produce an adult reproductive organism by artificial parthenogenesis. Though Delage obtained two sea urchins in this manner and more than one observer has so produced frogs, none of these reproduced a second generation, a fact not hard to understand on remembering that normally fertilized eggs of many animals have never been reared to maturity and reproductive activity under observation. Loeb considers a swimming larva to be the goal of the investigator. But it is interesting to note that the "swimming larvæ" of the marine worm *Chætopterus*, which he produced from unfertilized eggs, were shown by F. Lillie to be abnormal, unsegmented or poorly segmented eggs that had developed cilia.

We may consider for a moment what signifies development in the egg. The egg of any animal is in the beginning a single cell and undergoes a certain development before normal fertilization. Some animals reproduce parthenogenetically for several generations (*i. e.*, plant lice) and the silkworm eggs, noted before, undergo more or less development if fertilization fails to occur. The eggs of most animals, however, do not segment (produce an embryo) before fertilization. Though in many of these same species (for example, sea urchin) the eggs (during maturation) undergo before fertilization two very unequal cell divisions, resulting in the formation of "polar bodies," the unfertilized egg is generally considered a single cell, since the "polar bodies" seem to have no further part in the formation of the embryo. The positive sign of development in the mature egg seems to be segmentation. We may therefore consider artificial parthenogenesis to be demonstrated by the segmentation of unfertilized eggs which do not normally segment until fertilized.

R. Hertwig was the first to observe the segmentation of unfertilized

sea-urchin eggs, which normally die if not fertilized. Morgan, in 1899, produced segmentation in unfertilized sea-urchin (*Arbacia*) eggs, by immersing them in sea water to which a dry salt had been added, such a solution being called hypertonic sea water, and having a greater osmotic pressure than ordinary sea water. Loeb, immediately afterwards, produced swimming larvæ in sea water made hypertonic by the addition of magnesium chloride. Since then many investigators have studied the subject. Eggs of various animals have been made parthenogenetic by putting them in solutions containing salts, acids or alkalis, sugar, fat solvents, blood sera, alkaloids, or by means of asphyxiation, or by mechanical, thermal or electric changes. The concentration of the solution in which the eggs are treated may be the same as that of the fluid in which they normally live, or it may be of a greater or less concentration.

It has been fairly well demonstrated that the artificial agents—used in producing parthenogenesis—act primarily on the surface of the egg, and R. Lillie supposes they tend to increase its permeability. Loeb recognizes a “superficial cytolysis,” the exact nature of which is, however, unknown. When cells containing soluble coloring matters undergo cytolysis, the colored substances come out of the cells. Cytolysis has, therefore, been considered to consist of, or be accompanied by, an increase in permeability of the protoplasm.

The electric conductivity method of Kohlrausch suggested itself to the writer as the best way of settling this question of the permeability of the egg. The principle of the method lies in the fact that an electric current is carried through wet substances by the movement of electrically charged atoms or “ions.” If the permeability increases, the ions move faster and the current is greater. The use of this method showed that the permeability increased immediately after fertilization, on the application of agents producing parthenogenesis.¹ These results were confirmed by Gray,² who observed further that the permeability decreased again about fifteen minutes after fertilization. Lyon and Shackell³ observed that the permeability of the egg to certain dyes increased on fertilization, and also that more of the red substances came out of the fertilized than out of the unfertilized eggs. E. N. Harvey observed independently that the permeability of the eggs to certain dyes and caustic soda increased on fertilization. Finally, Glaser has recently concluded from his experiments that fertilization increases the egg’s permeability.

There is another proof that the egg of one species (the frog) becomes more permeable to salt on beginning development. The writer

¹ McClendon, *Am. Jour. Physiol.*, 1910, 27, 240.

² J. Gray, *Jour. Marine Bio. Assn. United Kingdom*, 1913, X., 50.

³ *Science*, 1910, 32, 249.

observed that the frog's egg may be made parthenogenetic by means of a momentary electric shock. Unfertilized eggs of a frog were divided into two equal lots, placed in distilled water, and one lot shocked electrically. It was found that three times as much salt diffused out of the shocked eggs as out of the control. Since the salt must have come from the interior of the eggs, the experiment seems to prove that the eggs must have been permeable to it. The shocked eggs began to segment and behaved in other ways as if normally fertilized.

There seems to be no doubt that the permeability of the egg is increased by agents producing parthenogenesis, but just how this influences the egg's development is not absolutely settled, because of the many processes in development which are very far from being solved. Some of these processes have been the subject of numerous investigations. The outward change in form during the segmentation of the egg is caused by changes in surface tension. Granted that fertilization alters the permeability of the egg, it may be that the changes in permeability influence the surface tension.⁴

The unfertilized eggs of sea urchins, frogs and many other animals are surrounded by jelly-like coats, the inner layer of which lies close to the egg. On fertilization, the jelly is pushed out by the perivitelline fluid exuding from the egg, the space occupied by the fluid being called the perivitelline space. The inner layer of the jelly looks like a distinct membrane and is called the "fertilization membrane." Loeb considers its formation of great importance. Biataszewicz has shown that the frog's egg shrinks as this fluid is "secreted." Glaser has observed the same phenomenon in the sea urchin's egg, though the shrinkage is so slight that other observers deny its taking place. Granting that the perivitelline fluid comes from the egg, the increase in permeability would facilitate its migration.

If the jelly be removed from the sea urchin's egg prior to fertilization, no "fertilization membrane" appears. Presumably the fluid is secreted but lost in the surrounding water.⁵ Though the membrane helps to protect the embryo, its existence is not absolutely essential, since eggs lacking it (due to the removal of the jelly) have been known to develop. Many observations and experiments have demonstrated to the writer that the tough "fertilization membrane" of the sea-urchin's egg does not exist (at least in its final condition) before fertilization. The increase in permeability allows the escape of the perivitelline fluid which, according to the hypothesis advanced in 1911, interacts with the jelly and forms the "fertilization membrane."⁶ Elder, in 1913, came

⁴ McClendon, *Bouss's Archiv*, 1913, 37, 233.

⁵ McClendon, "On the Nature and Formation of the Fertilization Membrane," *Internat. Zeit. f. Physik.-Chem. Biologie*, 1914, Vol. 163.

⁶ McClendon, *Science*, 1912, 33, 387.

to hold the same view. E. N. Harvey, though believing that the membrane is not present before fertilization, considers the jelly unnecessary for its formation, holding that the membrane substance hardens on contact with sea water. He admits that unfertilized eggs from which the jelly is removed soon lose their power of forming membranes on fertilization, but says they do not lose it immediately. Perhaps he left a thin film of jelly adhering to the eggs or had not removed the water containing the dissolved jelly. This dissolved jelly may be in time decomposed by bacteria and thus prevent membrane formation. If eggs with jelly remain in sea water fifty-two hours, they do not form membranes on fertilization.

When a sea urchin's egg is fertilized, an increase in the rate of respiration occurs, as shown by O. Warburg. This may be due to some physical change, and is to be expected, since the egg passes from a state of inactivity to one of activity. When the starfish egg is liberated from the body of the female into the sea, it becomes active to the extent of extruding the polar bodies. Loeb and Wasteneys found that respiration was high at the time of formation of the polar bodies in the starfish egg, and continued about the same level, whether fertilized or not. The egg may pass through an inactive stage while in the ovary, with corresponding low respiration. Coming in contact with sea water may stimulate it toward development, with resulting maturation and increased respiration, though the stimulus is not sufficient to cause segmentation. This is in harmony with the fact that much weaker stimuli cause segmentation in starfish than are required by sea urchin eggs. The frog's egg resembles the former and Batallion has shown that the slight prick of a needle is sufficient to cause the frog's egg to segment, while needles have been thrust by the writer all the way through sea urchin's eggs without causing either segmentation or death.

O. Warburg has shown that the respiration of all developing eggs is high, regardless of the methods used to cause segmentation. Respiration is therefore essential to development. Cleavage once started may be slowed or stopped entirely without materially decreasing respiration, indicating that respiration is not a result of cleavage. In order to discuss the relation of respiration to development, it is necessary to go more into detail on the general question of respiration.

OXIDATION OR CELL RESPIRATION

As is well known, the heat of a flame is unnecessary for the burning (oxidation) of many substances. For example, coal oxidizes slowly in the air, decreasing in weight, a fact which has led to efforts to preserve its fuel value by keeping it under water. Naturally, even slow combustion generates heat, and if the heat be confined, results in spontaneous combustion, *i. e.*, the raising of the temperature to the flame point.

Many substances burned in the body—sugar, for example—may undergo slow oxidation in alkaline solution in the presence of atmospheric oxygen (O_2). In the body, however, it is burned at a faster rate, leading to the conclusion that some other substance or substances are necessary. The search for such substances has led to the discovery of so called oxidizing enzymes, which oxidize many organic substances. It is characteristic of an enzyme, however, that it accelerates but one reaction. For the complete oxidation of grape sugar, for instance, it is supposed that a series of enzymes is necessary. This must remain for some time a supposition, as no pure substance or mixture of soluble substances has been extracted from the body that will completely oxidize grape sugar.

It might be concluded that "life" is essential to such oxidations, but such is not the case. In some instances ground up tissue, free from entire cells, absorbs oxygen and gives out CO_2 at a rapid rate. It is evident that some substances are completely oxidized in the process. The question has been raised as to whether the cell structure which has not been completely destroyed in grinding the tissue be necessary for the oxidation. In certain experiments Harden and McLean failed to observe respiration in juice pressed out of muscles and other tissues. Warburg and Meyerhof ground nucleated red-blood corpuscles with sand, finding that the mass did not absorb oxygen or give out CO_2 , whereas the original cells did. Warburg tried to destroy the structure completely by grinding corpuscles in a steel box; with steel spheres rotating at such high speed it was found necessary to cool the box with ice in order to prevent injury to the corpuscles by heat (Barnard & Hewlett apparatus). All microscopic structure was destroyed and respiration ceased.

In other experiments, Warburg ground up liver cells, passing the juice through a Berkefeld filter. The respiration of the juice was but five per cent. of that of the corresponding amount of liver cells. But when a coarser filter was used which allowed the passage of cell granules, the oxidation was found to increase to twenty per cent. of that of intact cells.

If blood corpuscles be placed in water, or in certain solutions, the hemoglobin passes out of them, they become pale and are called "ghosts." This liberation of the hemoglobin, known as "laking," is a kind of cytolysis. Warburg laked nucleated red-blood corpuscles of a goose, finding that respiration continued in the "ghosts," but did not occur in the fluid procured by laking.

Such experiments seem to show that the presence of solid structures, granules, etc., accelerates the respiration, since no substances were eliminated in the process of grinding. It is possible that the solid structures act in the same way, as does finely divided platinum (called platinum

black), which accelerates certain chemical reactions by the condensation of the reacting substances on the surface of the platinum, and their consequent increase in concentration. This process of condensation on surfaces is called adsorption. Warburg supposes that the oxidizing enzymes, oxidizable substances and oxygen are condensed on "surfaces," thus causing the oxidation rate to increase, but what surfaces he means it is difficult to determine, in some places apparently referring to surfaces of granules or colloidal particles, in others to cell or nuclear surfaces.

The adsorption of easily adsorbed substances may retard or prevent entirely the adsorption of others less readily adsorbed. Warburg found that anesthetics reduced the respiration of a mass of cell granules, presumably by driving the enzymes or oxidizable substances from their surfaces. He further observed that animal charcoal in water oxidized oxalic acid to CO_2 , whereas if anesthetics were added the oxidation was reduced.

Warburg and Meyerhof found that the respiration of sea-urchins' eggs was not entirely destroyed by grinding with sand, presumably because the cell granules were left intact. They explain it, however, as an auto-oxidation or spontaneous oxidation of lecithin in the presence of iron salts, the oxidation taking place in the test tube. Warburg found iron and lecithin in the sea-urchin eggs and observed that if the total lecithin that could be extracted from a mass of eggs were mixed with a dilute solution of iron chloride, the oxidation was as great as that of the mass of ground cells. From his data we conclude that the mass of ground unfertilized eggs undergoes the same oxidation as does the same mass of cells if it were fertilized before grinding. Warburg interprets this as indicating that the oxidation of unfertilized eggs is due to auto-oxidation of lecithin, and that the increase in oxidation on fertilization is due to increase in structure (surfaces). Since mechanical agitations, however, may cause the eggs to develop, it is possible that the grinding first stimulated each egg to as great respiration as that of a fertilized egg, but the crushing and subsequent mixing of substances reduced the oxidation. It is interesting to note that, whereas unfertilized as well as fertilized eggs absorb oxygen and give off CO_2 , ground eggs or lecithin and iron mixtures do not give off CO_2 , indicating oxidation is not complete.

RELATION OF OXIDATION TO PERMEABILITY

R. Lillie supposed the oxidation within the unfertilized eggs to be suppressed by an accumulation of some end product of oxidation that could not escape. It is possible that such a substance might act like an anesthetic and suppress oxidation by adsorption to the granules. Lillie supposed this substance to be carbonic acid, but this is hard to

believe when we bear in mind that the egg may be caused to develop by short exposure to carbònic acid. On fertilization this hypothetical substance would be liberated and could be collected. Glaser fertilized quantities of eggs in a small amount of sea water. On using the same water in which to develop other fertilized eggs, he found it inhibited their development, indicating the presence of an inhibiting substance that came out of the first eggs on fertilization. (Was this CO_2 ?)

Loeb's "improved method of artificial parthenogenesis" claims two treatments of the eggs to be necessary. They are first to be stimulated to development by use of fatty acid, or some other method, and then exposed to a hypertonic solution. The latter he calls a "corrective agent" and supposes that it changes the character of the oxidation in the egg, since he observes no effect on the rate of oxidation in the developing eggs. It is hard to conceive of such a change in "character," since oxidation means union with oxygen and there is but one kind of oxygen atom in combinations. The oxygen might attack different substances, but in such cases different amounts of heat would be given off, the heat of combustion of fats and carbohydrates, for instance, differing in amount. Meyerhof showed that the ratio of oxygen used to heat produced was the same for eggs in the hypertonic solution as in sea water. When we consider that by the use of either fatty acid or hypertonic solution alone, sea-urchin (*Arbacia*) eggs may be made to develop, it seems unnecessary to devote more time to their combined effect.

RELATION OF ANESTHESIA TO DEVELOPMENT OF THE EGG

Anesthetics have a depressant action on various cell activities when used in certain concentration. They decrease the respiration and rate of cleavage of sea-urchin eggs (and asphyxiation will cause cleavage to cease). It may therefore be supposed that it is the suppression of oxidation by anesthetics that suppresses cleavage. Warburg, however, caused the almost complete cessation of cleavage in sea-urchin eggs with anesthetics without appreciably lowering the respiration. It may be that the anesthetic acts in one part of the cell (on the surface of the granules) in suppressing oxidation, and in another (on the cell surface) in suppressing cleavage.

In 1909, while measuring the electric conductivity of sea urchins' eggs, the writer observed the decrease in conductivity on the addition of a certain per cent. of chloroform. This experiment was not repeated, but we may imagine that the chloroform decreased the permeability of the eggs to ions. Osterhout, becoming interested in the methods used, modified them for use with plants, and observed a decrease in electric conductivity of certain plants (kelp) when using a certain concentration of anesthetic, indicating that the anesthetic decreased permeability. R. Lillie found that anesthetics might antagonize the action of the pure

salt solutions used to cause eggs to develop, presumably preventing the increase in permeability usually caused by the salt solution.

The use of fish eggs in settling this question presented itself to the writer. It was found that the eggs of the pike will develop in distilled water and are practically impermeable to salts—that is to say, that the salts which they contain diffuse out of them only in such small quantities as to render detection almost impossible even with as sensitive an instrument as the nephelometer. It was found, further, that pure solutions of sodium nitrate increased the permeability of the eggs to chlorides (since the chlorides diffused rapidly from the eggs). The use of anesthetics prevented the effect of nitrates on the permeability of the eggs, so that the chlorides failed to diffuse.⁷

It is thus evident that the problem of parthenogenesis is closely interwoven with fundamental problems of physiology—stimulation, oxidation and anesthesia; and that the final elucidation of parthenogenesis and fertilization must wait on the solution of these other problems. On the other hand, the systematic study of parthenogenesis has already shed much light on general physiology, and progress will be more certain if all of these problems be kept before the mind of the investigator.

⁷ *Science*, 1914, Vol. 40, p. 214.

THE PRESERVATION OF THE LIFE OF THE FROG'S EGG AND THE INITIATION OF DEVELOPMENT, BY INCREASE IN PERMEABILITY

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Received for publication April 29, 1915

If the frog's egg is removed from the body of the female and placed in water it soon dies and disintegrates. This is true whether the egg is removed from the ovary, the body cavity or the oviduct. Even though the egg were laid in the normal fashion, it would die if not stimulated by a spermatozoon or in some other manner. Bataillon² was able to cause unfertilized frog's eggs to segment by pricking them with a needle. But I (1) found that a much more convenient and efficient method of causing them to segment is to stimulate them with the ordinary 110-volt alternating current from carbon or platinum electrodes placed about two inches apart in the water containing the eggs, for about one second. Bataillon thinks the introduction of a foreign protein, such as is used to induce anaphylaxis, is necessary for the development of these eggs. I found the jelly in which the eggs are imbedded to be permeable to hemoglobin, and it is possible to think of protein diffusing in without the needle prick. But eggs removed so carefully that there is no possibility of foreign protein gaining access to them, segment when stimulated electrically. It is certain that no foreign protein is necessary for the beginning segmentation of this egg,

¹ The nephelometer used in this work was bought with a grant from the Research Fund of the Graduate School.

² Bataillon: *Comptes Rendus de la Société de Biologie*, 1911, lxx, 562.

though it may be necessary for its later development. In the leopard frog, *Rana pipiens*, this segmentation usually stops before the first cytoplasmic division is completed, although nuclear divisions may continue for several days.

The initiation of cleavage in the frog's egg by the simple prick of a needle, which I have observed in American frogs, might be interpreted as increased permeability. Since I have shown that electrical stimulation of muscle causes increased permeability (2), electrical stimulation of these eggs might be supposed to have the same effect. In order to determine this point, I made some preliminary experiments last year, but owing to difficulty in obtaining material, could offer but meagre data to prove the point (3). This year the leopard frogs bred in the laboratory, and furnished abundant material.

A large number of females of the same size, and that were clasped by males, were placed in a tank with two inches of water. As soon as a female deposited her eggs, these were carefully removed to distilled water. Two females were opened and their eggs placed in separate dishes of distilled water, one as a control and the other for electrical stimulation.

In removing the eggs, the female was washed free from sperm, the head cut off, most of the viscera removed and the blood washed out. The distended oviducts were carefully removed and the blood washed out of the vessels in their walls. A hole was made in each oviduct and it was dragged across the floor of a dry glass dish, leaving a trail of adhesive eggs. When distilled water was added the eggs remained adhering to the glass.

In order to guard against slight differences in the masses of eggs, the two oviducts of one female were emptied in separate dishes and one used as an unfertilized control and the other stimulated electrically. The batch of fertilized eggs of another female was cut in two equal parts, and one part placed in a third dish.

The eggs were washed in repeated changes of distilled water for an hour. During this time the gelatinous coverings of the eggs partially swelled. The sperm traverses the jelly and reaches the inseminated eggs in about one hour, and the eggs rotate until their black poles are turned upward. It is now time to stimulate the unfertilized eggs in one dish. Two clean platinum electrodes, about one inch apart are passed along opposite sides of each row of eggs, until all of the eggs in the dish have been stimulated. These eggs now begin to rotate, and some of them are completely rotated in one minute. If the rows of

eggs are slender so that the jelly is uniformly swollen, all of the eggs rotate at about the same time. None of the eggs in the unfertilized control rotate, no matter how much the jelly swells. This is due to the fact that the jelly sticks tight to the egg, whereas when they are stimulated, a fluid is secreted between the egg and the jelly.

The water is now poured or syphoned off of all the eggs and a measured quantity added to each dish. Ordinary distilled water was boiled and redistilled in an automatic quartz apparatus (4) and used in the experiments. At the end of certain intervals of time, the water in each dish is stirred, and 25 cc. removed for analysis of chlorides with the nephelometer. At the end of seven hours all of the water is removed from all of the eggs. An aliquot part of it is evaporated in quartz for ash. Water is added to all of the eggs and another series of determinations made.

The following table gives a sufficient number of the results to indicate their character.

NO. OF OVIDUCTS TO EACH DISH	cc. H ₂ O	CC. OF A $\frac{1}{100}$ NORMAL CHLORIDE SOL. IN H ₂ O			GRAMS ASH IN H ₂ O
		0-3.5 hrs.	0-7 hrs.	7-30 hrs.	0-7 hrs.
1 unfertilized.....	500	0.70	1.20	0.38	0.005
1 stimulated.....	500	1.40	2.24	1.22	0.008
1 fertilized.....	500	1.22	2.20	1.20	0.007
2 unfertilized.....	1000	1.40	2.40	1.42	0.015
2 stimulated.....	1000	1.90	3.60	2.40	0.024
2 fertilized.....	1000	1.80	4.20	2.00	0.023
4 unfertilized.....	2000	2.80	4.80	3.00	0.031
4 stimulated.....	2000	4.00	7.60	4.80	0.053
4 fertilized.....	2000	3.80	7.20	4.20	0.045

The first column shows the number of oviducts used in supplying each dish with eggs, and the second gives the number of cc. H₂O added. In the first 3.5 hours as much chloride diffused out of the eggs as in 23 hours, beginning with the eighth hour. The table gives the total quantity of Cl in the H₂O as calculated from analysis of a 25 cc. sample, and the result may be converted into grams by multiplying by 0.035.

In obtaining the ash, three-fourths of the H₂O was evaporated down in quartz beakers. During the evaporation the foam was skimmed off and transferred to a platinum crucible. When the volume of the water was reduced to a few cc. it was transferred to the crucible. The

ashing was done at as low a temperature as possible by admitting air into the crucible through a small metal tube while it was heated. Some chlorides may have been lost, but the results are comparative. The ash was found to contain about 60 per cent of insoluble salts and 40 per cent of soluble salts. The ash contained Na, Li, K, Ca, Mg, Cl, SO_4 and CO_2 , but no phosphates were detected. The metals were detected in the flame spectrum and the spark spectrum (fulgurator). Mg was also detected by means of the displacement of the bands of alkanin. Na was also determined quantitatively as sodium uranyl acetate and as silicofluoride but the quantities were too small to be accurate. SO_4 was precipitated with BaOH, and CO_2 driven out of the dry ash with acid.

The diffusion of salts from the electrically stimulated eggs is about the same as from the fertilized eggs, but nearly double that from the unfertilized eggs. This difference was found to continue for 50 hours, when the determinations were discontinued as the unfertilized control had died.

In making prolonged experiments it is important to change the water frequently in order to keep down the bacteria, which might bind the salts or interfere with the nephelometer reading. In previous years, I have charred and extracted the samples before nephelometric determinations, but think it a bad practice and only necessary in the presence of bacteria or protein. The traces of dissolved glyco-protein from the jelly did not cause a measurable error as a great excess of AgNO_3 (5 drops of a saturated solution) was added to the 25 cc. sample, and the reading was taken in one minute, whereas several hours are required for perceptible reduction of silver by these solutions in the intensity of light to which they were exposed. I found it advantageous to use open tubes closed at one end with black rubber stoppers in the nephelometer, in order to avoid the reflection from the bottom of test tubes, but this was not absolutely necessary with the Richards nephelometer, because a part of the large field shows no reflections, and test tubes can be more easily cleaned.

The supposed relation between increased permeability and cleavage has been fully discussed in a previous paper (5) in which I showed that an increase in permeability of the sea urchin's egg was produced by any treatment which causes cleavage.

The fertilized or stimulated frog's egg may live a long time, whereas the unfertilized frog's egg soon dies when placed in water. The question arises whether the increased permeability saves the life of the egg.

In order to decide this we must first consider the effect of water on the egg.

The frog's egg swells when placed in water, and I found that the unfertilized egg swells more than the fertilized or stimulated egg, until the death of the former terminates the physiological comparison. In eggs of the same female, the mean diameter of 23 unfertilized eggs 30 minutes after being placed in water was 1.52 mm. whereas the mean diameter of 23 that had been stimulated by the electric current and left in water 30 minutes was 1.47 mm. After cleavage begins the estimation of the volume of the eggs becomes more difficult, and on the formation of spaces between the cells of the fertilized egg the apparent volume is much greater than the real volume of the protoplasm. But I think that one effect of fertilization is clearly the retardation of the swelling of the egg. One need only suppose that the death of the unfertilized egg is caused by rapid swelling, in order to explain the life-saving action of fertilization or stimulation. Bachman found the unfertilized salamander egg bursts in two to seven hours if placed in water but not if in salt solution. It is not so easy to determine the exact moment of death of the frog's egg.

The retardation of the swelling of the fertilized or stimulated egg is evidently due to the loss of osmotic substances, such as the salts which I have given in the above table. At the moment of increase in permeability, a concentrated solution of these substances passes from the egg and pushes out an adhering membrane which we may call the fertilization membrane. Bialaszewicz³ claims that a measurable decrease in volume of the egg takes place at this time. We may take this decrease in volume of the egg to equal the volume of the secreted fluid, but after this perivitelline fluid has once been secreted it increases by absorption of water through the fertilization membrane. I found this membrane to be easily permeable to salts since it cannot be easily plasmolyzed (shrunken) by immersion in hypertonic salt solutions. Hence it is probable that the perivitelline fluid contains other less diffusible substances. An analysis of the perivitelline fluid of the *Amblystoma* egg showed that it contained salts and organic substances but only a trace of protein. The perivitelline fluid of the egg of the giant salamander contained 0.16 per cent of dissolved substances. Backman and Runnstrom⁴ suppose these substances to be chiefly secreted by the suckers that develop on the head of the frog embryo,

³ Bialaszewicz: Bull. Acad. Sc. Cracow, Math.-Nat., Oct. 1908.

⁴ Backman and Runnstrom: Pflüger's Archiv, 1912, cxliv, 313.

but this is hardly in harmony with the fact that the salamander embryos have no suckers.

The function of the perivitelline fluid seems to be to make room for rotation of the egg and extension of the embryo, by pushing out the fertilization membrane, which is chiefly effected by the osmotic pressure of the dissolved substances that it contains. The loss of salts must be more or less independent of this, since salts are lost continuously during development, and salts would not be very effective in pushing out the membrane since it is permeable to them.

This loss of salts explains the results of Backman and Runnstrom⁵ who found that the osmotic pressure of the frog's egg is reduced when it is fertilized and placed in water. They attempt to explain it, however, by erroneously assuming that fertilization causes a coagulation of the proteins and that the coagulation adsorbs the salts. They seem to consider this egg as a diphasic system in which the watery phase forms the main bulk of the egg. On the contrary, I found the cytoplasm to be a 4-phase system in which the watery phase is a very small fraction of the total volume (6). The salt would have to be lost from this watery phase only, in order to reduce the osmotic pressure of the egg, and the more permeable the plasma membrane is to them the less osmotic pressure they can exert while in the egg. I found the watery phase to be 16 per cent of the whole egg and to contain 82 per cent water (7). These figures are a little too high owing to slight admixture of other phases, but after removal of these and of the dissolved proteins, the water-soluble substances in this layer formed only 1.7 per cent of the weight of the egg.

The chlorides diffusing out of the egg in seven hours would make a solution in the watery phase more concentrated than $\frac{1}{100}$ normal and the total salts diffusing out in this time would form nearly 1 per cent of the watery phase. Since the osmotic pressure of the ovarian egg corresponds to a 0.166 normal solution of NaCl and is probably lowered in the passage of the egg through the oviduct, the increased permeability and diffusion seem to be sufficient to account for the failure of the egg to die in the very hypotonic water in which it develops.

By an analysis of the ovarian eggs, I found that the chlorides were not sufficient to raise the osmotic pressure of the watery phase to equal that of frog's blood or a 0.166 normal NaCl solution, and that they were not bound during coagulation of the protein (furthermore there is

⁵ Backman and Runnstrom: *Biochem. Zeitsch.*, 1909, 390.

These salts are not entirely without toxic action, for this is manifested in the swelling of serous cavities. The swelling of the pericardium is greater in lithium solutions than in isotonic solutions of Na or K. In more concentrated solutions, the individual cells separate from one another, a condition which Roux called *framboisea* of the embryo.

A marked contrast exists in the effects of pure salt solutions on the eggs of the frog and those of many fish. These fish eggs are normally impermeable to salts and water and the osmotic effects are never observed. The toxic effects of salts are, however, more manifest (11). These toxic effects, resulting in the swelling of serous cavities and other abnormalities, are associated with increase in permeability (12). Though a slight increase in permeability does no damage, a greater increase causes abnormalities and a still greater increase causes death. It is probable that the swelling of the pericardium of the frog's egg is produced by increase in permeability in the same way as the swelling of the pericardium of the fish egg. The difference between the two is that, whereas the fish egg is normally impermeable to salts and to water, the frog's egg is permeable to water and to a less degree to salts. Fertilization increases this permeability to the optimum degree, and pure salt solutions increase it too much.

SUMMARY

Fertilization or electric stimulation increases the permeability of the frog's egg so that Na, K, Li, Mg, Ca, Cl, SO_4 and CO_2 diffuse out at a faster rate. Probably the swelling of the pericardium, due to the action of pure salt solutions, is caused by too great increase in permeability. Notwithstanding the outward diffusion of salts, the egg absorbs water, and the addition of osmotic substances to the medium, preventing the absorption of water, prevents the segmentation of the white hemisphere, and gastrulation.

The abnormally rapid swelling of the unfertilized egg probably is the cause of its death, and thus the increase in permeability on fertilization lowers the osmotic pressure and saves its life.

The low osmotic pressure of the egg observed by Backman and Runnström was probably caused by three processes: (1) The lowering of the osmotic pressure as the egg descends the oviduct; (2) the increased permeability and loss of salts; (3) the admixture of jelly of low osmotic pressure with the eggs in taking the freezing point.

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**THE COMPOSITION, ESPECIALLY THE HYDROGEN
ION CONCENTRATION, OF SEA WATER IN
RELATION TO MARINE ORGANISMS**

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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXVIII, No. 1, DECEMBER, 1916

THE COMPOSITION, ESPECIALLY THE HYDROGEN ION CONCENTRATION, OF SEA WATER IN RELATION TO MARINE ORGANISMS.

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(Received for publication, September 14, 1916.)

Since the extensive analyses of Forchhammer and Dittmar, the relative concentration of the chief salts in sea water has been accurately known, the differences in the results of different investigators only slightly exceeding the differences in the values of the atomic weights that they used. Omitting regions affected by land drainage and the melting of the polar ice caps, ocean surface salinity (gm. of total salts per kilo) varies only from about 33 to 38, the most dilute region being the North Pacific. The known ocean currents affect only the surface layers, but there is probably a drift of the bottom water chiefly from the South and less from the North Polar regions toward the equator, which could take place at a depth of 4,000 meters without obstruction. The evidence for this is the fact that the depths of the oceans have a remarkably uniform salinity of nearly 35 and temperature of about 2° which is much above the temperature of maximum density, and are fairly well oxygenated. It seems safe to infer, therefore, that all surface water has comparatively recently come from the depths under the equator.

Dittmar showed that there is proportionately 0.44 per cent more CaCO_3 at the bottom than at the surface, due to the solution of pelagic shells by means of the CO_2 from organic decomposition. It is evident, therefore, that the CaCO_3 brought from the depths and from the land is precipitated in surface water, especially in the tropics. Drew supposes that this is not done entirely by the formation of shells and skeletons, but also by the action of denitrifying bacteria which increases the alkalinity of the water by removal of nitrates until the solubility product for CaCO_3 is exceeded. Palitzsch has shown that the alkalinity of the sea varies inversely with the depth, but this is probably largely the result of changes in CO_2 content due to the synthesis of organic matter at the surface and its decomposition in the depths, and less to the changes in nitrates, especially since nitrates and ammonia vary in the same direction. The effect of denitrifying bacteria is probably greatest where the sea is diluted with fresh water. I

found that CaCO_3 began to precipitate on the glass when the $P_H (= -\log H^+ \text{ concentration})$ was kept at 8.26, although the addition of CaCl_2 causes no precipitation.

In selecting a place to study sea water, the coast laboratories are to be avoided, owing to the drainage of the land. Therefore it was decided to go to Tortugas, Florida, where the water may be considered ocean water since it is a deep blue with an average salinity of 36 (35.23 to 36.09) (Dole) and is remarkably free from plankton or sediment. The scarcity of life is probably due to the lack of fixed nitrogen as I found 0.0085 mg. of NH_3 in a liter and hardly a trace of nitrates in 2 liters. Since Tortugas is about 500 miles from the mouth of the nearest large river, the accession of fixed nitrogen from the land is negligible, and its marine flora is dependent on that coming up from the depths or down with the rain, which escapes the action of the denitrifying bacteria.

The temperature of the Tortugas laboratory (which is practically in the sea) showed a diurnal rhythm of about $25\text{--}31^\circ$, and a general drift of about 2° during the season. Most of the experiments were done at a temperature of $29.75\text{--}30.25^\circ$. The temperature of the sea was not taken regularly, but it was always near 29° .

The special Leeds and Northrup potentiometer (reading to 0.5 millivolt), Weston cell (of the unsaturated type), and weights (used in making the solutions) had just been standardized by the United States Bureau of Standards. The calomel electrodes were made as follows: A round-bottomed 100 cc. cylinder, with a side neck attached near the top and bending down to the bottom, was supplied with a large platinum electrode fused in the bottom. Redistilled mercury, purified by shaking in a shaking machine for 30 minutes with each of several changes of 3 per cent nitric acid, was dissolved in nitric acid, and this mercurio-nitrate was used in making the mercury by electrolysis. The electrode was filled with the mercurio-nitrate and the side neck dipped into a vessel of mercurio-nitrate containing a platinum anode. A mercury anode was tried and discarded since I could not find a method of preventing the formation of a less soluble mercury salt on its surface. As much current was passed, through a resistance, from the 110 volt direct current circuit as was possible without undue heating of the side neck, until the platinum was plated over its whole surface with a thick layer of mercury, and mercury commenced to drop off of it in considerable amount. The KCl was recrystallized by solution in hot distilled water in fused quartz and cooling (and throwing away the mother liquor) five times, and dried 3 months over CaCl_2 . The solution was made as needed by weighing out 7.456 gm. and dissolving it in

distilled water in a liter flask. A fresh portion of mercurio-nitrate was purified by electrolysis, redissolved, and precipitated with Baker's analyzed HCl, and the precipitated calomel washed by decantation several times with distilled water and many times with the 0.1 N KCl, always being in contact with excess Hg which was finally shaken to form a gray mixture with the calomel, and washed into the electrode with KCl solution until the platinum was deeply covered. The end of the side neck was closed with an ungreased ground glass cap and passed through the rubber stopper of a cylinder filled with 0.1 N KCl. The electrode was filled with 0.1 N KCl and closed by fusing the glass in a flame. The second cylinder was connected by means of a syphon closed with an ungreased ground glass cap to a third cylinder filled with a saturated solution of KCl. The electrode vessel and part of the side neck were painted black to prevent the reduction of the calomel by light.

The only course left for change of this electrode is change in average size of grain of calomel, due to the greater solubility of the smaller grains, but it seems to be a general opinion that such a process is very slow. The electrodes measured zero against one another when first made up and when they were periodically tested later. They gave the expected E. M. F. against electrodes of several other types. Since I aim at an absolute accuracy of 1 millivolt ($\pm 0.016 P_H$), their drift of potential during the season cannot be considered serious.

The combined tonometer and hydrogen electrode and the improved hydrogen electrode previously described (McClendon and Magoon) were used, with the exception that the latter was made with the large compartment about 11.5 cc., owing to the fact that the buffer value of sea water is less than that of blood. After passing the hydrogen bubble over into the 1.5 cc. compartment the end cock (d) was opened before closing the middle cock (c) in order to restore the pressure that had been reduced by the solution of some hydrogen. Several forms of titration hydrogen electrodes were tried but the one shown in Fig. 1 is to be recommended, owing to the absence of rubber bulb, piston, or mercury funnel to suck up the liquid into the electrode. It is simply immersed in the liquid far enough so that when the cock is properly turned the liquid enters the electrode and drives some of the hydrogen out through the trap T. It is likewise preferable to other non-sucking titration electrodes in being more protected from diffusion of O_2 from the fluid outside.

The hydrogen electrode proper in each of these instruments was made of a gold disc (welded to platinum wire) cleaned with a saturated solution of potassium bichromate in concentrated sul-

furic acid, washed, and platinized with 2 per cent platonic chloride containing a trace of basic lead acetate. It was not cleaned by electrolyzing H_2SO_4 , since it was never used as anode and the possibility of the formation of Cl_2 was excluded. It was replatinized a few seconds after each dozen determinations. Since this process would finally make the platinum black too thick, the life of the electrode may be prolonged by the use of palladium, which can be dissolved off (Clark and Lubs; Ostwald and Luther).

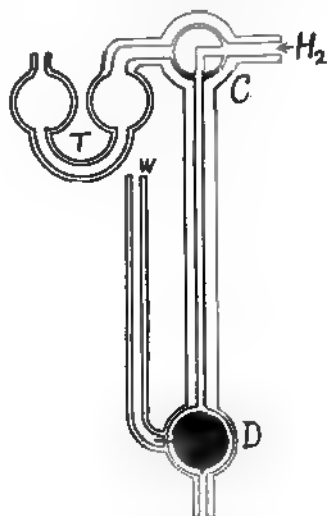


FIG. 1. Hydrogen electrode for titration. Insert a drop of Hg and a copper wire in W. The bulb, D, is immersed in the fluid in a beaker and hydrogen is bubbled through it. The cock, C, is turned so that the bulb fills (by gravity) with fluid which drives hydrogen out at T. Hydrogen is bubbled through again and the fluid allowed to rise until it just touches the platinized gold disc (black), when the reading is taken.

The hydrogen was generated from zinc and H_2SO_4 and washed with (1) alkaline permanganate, (2) $HgCl_2$, and (3) alkaline pyrogallol, in all glass, stopperless wash bottles. The CO_2 was generated from marble and HCl and washed with $NaHCO_3$ solution.

Owing to the high electrical resistance in the circuit caused by the 0.1 N KCl in the two ground joints in the calomel electrode (which were always closed) and the sea water in the ungreased stopcock of the hydrogen electrode, which was also closed, the

ordinary capillary electrometer was not sufficiently sensitive to read down to 0.5 millivolt. The capillary tube of the electrometer was drawn out in the flame to about 0.01 mm. bore and until the wall was thin enough (even after pasting a cover-glass on it with Canada balsam) to admit the use of an 8 mm. objective and very high ocular in the reading microscope. By bending back the bulb of the capillary electrometer it was made to fit on the stage of a microscope tilted back and held by rubber bands. In order to prevent shaking, the table legs were passed through the floor into the ground without touching the floor. The use of the capillary electrometer saves much time.

The millivolt readings after correction for H_2 density, according to Clark and Lubs, were converted into P_H values by means of the conversion table previously published (McClendon, 1916) which was extended sufficiently for the purpose. This change makes all the values in the table about 1 millivolt too low, and therefore 1 millivolt was arbitrarily subtracted from each of Clark and Lubs' corrections. Clark and Lubs find a different theoretical value for the E. M. F. of the normal hydrogen electrode against the 0.1 N calomel electrode from that used in making the conversion table. Since much of the older work was done with HCl, and since Ellis has shown that its curves for dissociation calculated (1) from electrode potential and (2) conductance ratio are different even at considerable dilution, the discrepancy may be due to the use of the conductance ratio to denote the dissociation of HCl at dilutions at which it differs from that calculated from electrode potential.

Since my solutions were near neutrality and the reading was taken immediately after making junction with saturated KCl solution, I do not think that the diffusion potential vitiates my results.

For preliminary tests a series of "nonsol" glass tubes (1 cm. bore) filled with Sørensen's phosphate mixtures plus phenolsulfonephthalein from P_H ($= -\log H^+$ concentration) = 7-8, and a similar set of tubes filled with Sørensen's borate buffer mixtures plus thymolsulfonephthalein¹ (Lubs and Clark) from P_H = 8-10, and sealed in the flame, were used. The phosphate and borate mixtures were calibrated with the hydrogen electrode, but owing to the salt action on the indicator 0.3 must be subtracted from the P_H of sea water as determined with the tubes in order to obtain the same result as with the hydrogen electrode. Although the P_H of sea water was determined with the hydrogen electrode out to the second place of decimals

¹ For the convenience of Dr. Rowntree, Dr. A. G. Mayer, and others I have standardized solutions or samples in relation to the phenolsulfonephthalein and thymolsulfonephthalein tubes for determining hydrogen ion concentration kept in stock for sale by Hynson, Westcott and Co., Baltimore.

(0.5 millivolt = $0.008 P_H$), the estimation of the salt action on the indicator could not be carried this far because the tubes read only to the first place of decimals. Palitzsch defines the P_H to the second place of decimals, but this must be an estimate since his borate mixtures record only to the first decimal place. The apparently remarkable coincidence that the correction for salt action (at 35 to 36 salinity) should be the same for phenol-sulfonephthalein and thymolsulfonephthalein is probably due to the similar chemical constitution of these indicators. The advantage of these indicators over α -naphtholphthalein and phenolphthalein is that each shows two strikingly different colors, and errors due to dye concentration cannot be hidden.

These tubes of thymolsulfonephthalein are to be especially recommended for determinations at sea in oceanographic work and all determinations of the P_H of sea water in which an accuracy of 0.1 is sufficient. They were used by Dr. L. R. Cary to measure the CO_2 excreted by medusæ (see also Haas). For a greater accuracy the tubes would have to be made larger or of extremely uniform bore. In making the small tubes the glass should be carefully selected. The bore should be 10 mm. \pm 0.25 mm. They should be filled with 3 cc. of the buffer mixture plus 0.2 cc. of 0.1 per cent thymolsulfonephthalein in 70 per cent alcohol, measured with a graduated pipette. A test-tube of 10 mm. bore should have an etched mark at 3.25 cc. for use with sea water, since the addition of the sea water after the indicator helps mix the latter. By retaining the 0.2 cc. pipette used in filling the tubes, the necessity of the calibration of one for making the tests is avoided.

The P_H of Tortugas sea water (within 8 miles of Loggerhead Key or $82^\circ 52'$ to $82^\circ 58'$ W. and $24^\circ 30'$ to $24^\circ 38'$ N.) from the surface to a depth of 35 meters, as well as in the moat of Fort Jefferson, was found to vary from 8.1 to 8.22, which is about the average for ocean water (7.95 to 8.25, Palitzsch; P_H of 8.06 just colors phenolphthalein, at 35 salinity). Since there was no general drift of P_H during the season, it is probable that the limits of variation during the entire year are not very different. No relation between P_H and location, time, or tide was found.

Since calcium is the only non-volatile base in sea water that has been shown to vary independently of the salinity, it is possible that variations in the calcium content due to the activity of corals and other organisms might affect the P_H but this question must be reserved for future study. Since I found only 8.5 parts of ammonia per billion, this factor must be excluded. The effect of denitrification is probably small, since I found only minute traces of nitrates in the water. The amount of nitric acid brought down by the rain in the Barbados was estimated by Harrison and Williams at 2.75 kg. per hectare per annum, but this was accompanied by 1.13 kg. of ammonia, which would mean a mixture of NH_4NO_3 and NH_4CO_3 . After

thunder showers over the sea this fixed nitrogen is probably taken up very quickly by algae and denitrifying bacteria. Although the evaporation is rapid, it would not be sufficient to concentrate the fixed nitrogen before it mixed with the sea to a great depth, since Dole could detect no immediate influence of rain on the surface salinity.

That variations in the amount of H_2PO_4 in the sea water could account for variations in the P_n seems improbable, since I found about 4 mg. in 20 liters that had been acidified with HCl and evaporated to a small quantity. Although some phosphates may have been occluded in the salts crystallizing out, this fraction was probably small, owing to the strongly acid reaction which was constantly maintained during the evaporation.

Since dilution of the sea water must dilute the carbonates and bicarbonates responsible for its reaction, it would theoretically affect the P_n . Solutions of soda were aerated by drawing moist air through them vigorously for 72 hours in order to make their CO_2 tension equal that of the air. The P_n of the 0.1 N solution was 9.8; that of the 0.01 N, 9.26; and of the 0.001 N, 8.3. We thus see a marked change in the P_n with dilution, but the effect of diluting a bicarbonate solution is much less if it is not aerated; we cannot say that the sea is thoroughly aerated, and, furthermore, the presence of neutral salts in the sea affects the dissociation of the alkali. In order more nearly to imitate the effect of rain falling in the sea, a portion of sea water of $P_n = 8.1$ was diluted with an equal volume of conductivity water of $P_n = 6$, and the P_n of the mixture was found to be about 8.09 in the hydrogen electrode. Perhaps if the CO_2 tension of the conductivity water had been exactly that of the air, a greater change would have been noted, but the experiment indicates that a dilution of the most concentrated Tortugas sea water (salinity = 36.09) to the most dilute (salinity = 35.23) would not account for the variation in P_n (from 8.22 to 8.1).

There remains to be considered the change in CO_2 content and its effect on the P_n of the sea water. No direct measurements of the CO_2 content of the sea water were made, but the change of P_n with change of CO_2 tension is shown in Fig. 2. In making mixtures containing less than 1 per cent CO_2 , a double dilution is necessary. For instance, in making 0.02 per cent CO_2 a 1 per cent mixture is made in the combined tonometer and hydrogen electrode as previously described (McClendon and Magoon), then the apparatus is shaken (without disconnect-

ing the mercury funnel) so as to mix the CO_2 thoroughly with the H_2 . By raising the mercury funnel, all but 2 per cent (2 cc.) of the gas mixture is expelled, then hydrogen is admitted in the usual manner so that the resulting mixture contains 0.02 per cent

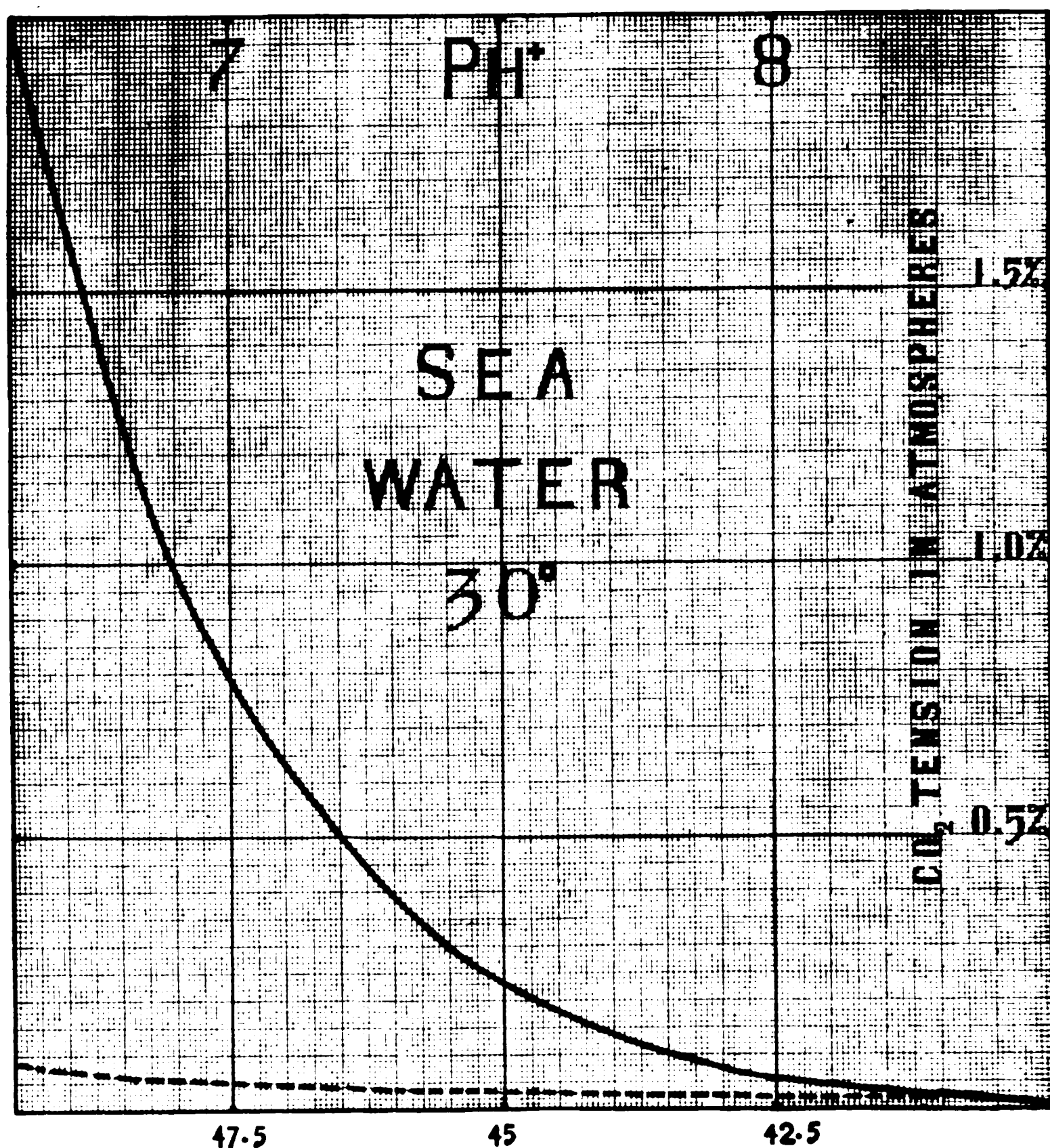


FIG. 2. Table for converting P_H of sea water (35 salinity) into CO_2 tension and total CO_2 content at 30° . The dotted line represents cc. of total CO_2 per liter.

CO_2 . After shaking 1.5 cc. of sea water with 10 cc. of a gas mixture in the tonometer, the CO_2 content of the gas mixture may be perceptibly changed, so it was necessary, after determining

the P_H , to expel the gas mixture and make a new one like it, and repeat this process until a constant P_H was reached.

The change in P_H with a change in CO_2 tension is shown in the continuous line in Fig. 2. The dotted line shows the total CO_2 content extrapolated from data given by Fox, and is 40 cc. at 0.015 per cent CO_2 tension, 41.9 at 0.02 per cent, 44.5 at 0.03 per cent, 46.25 at 0.04 per cent, and 47.5 at 0.05 per cent.

Fig. 2 shows that within the normal limits for sea water a very small change in the CO_2 tension or CO_2 content causes a comparatively large change in the P_H , and the changes that have been observed in the P_H of uncontaminated ocean water could probably be explained by the changes in CO_2 tension produced by the action of organisms. I found that a jellyfish pulsating in sea water in a closed vessel changed the P_H from 8.2 to 8 in a very short time, indicating an increase of about 6.5 cc. of CO_2 per liter. Algæ in the sunlight would change the P_H to 8.25 in less time. It seems almost certain that the CO_2 tension is the chief factor in determining the P_H of the oceans. If this is true, the water from a depth of 2,000 meters in the Atlantic, which Palitzsch found to have a P_H of 7.95, should have a CO_2 tension of about 0.06 per cent at 30° , and Tortugas water should have a CO_2 tension of about 0.03 to 0.043 per cent. In one experiment water drawn directly from the sea into the electrode had a P_H of 8.15. It was then passed into the tonometer and its P_H at different CO_2 tensions determined, with the result that it had a P_H of 8.15 at 0.04 per cent CO_2 tension, from which I conclude that this was the CO_2 tension of the sea where the sample was taken.

It would be interesting to know the CO_2 tension of the air at Tortugas to see how it compared with the sea. An attempt was made to determine this by bubbling air through the water until equilibrium was reached and then determining the P_H of the water. The first experiments were valueless, owing to the assumption that equilibrium would be reached within a few hours. It was found necessary to pass a strong stream of moist air through a small quantity of sea water at least 15 hours in order to reach equilibrium, and during this time the temperature changed about 5° if the aeration was all done continuously. In one experiment starting with $P_H=8.1$, equilibrium was reached at the end of 15 hours' aeration with the final temperature 30° , and $P_H=8.16$. After 24 hours' more aeration (which was stopped at a lower temperature), the P_H was about the same (8.15).

We may assume, therefore, that the CO_2 tension of Tortugas air on that day was about 0.033 per cent. Legendre found the air off the coast of France to contain 0.022 to 0.087 per cent CO_2 , and Lewy found ocean air in the Antilles to contain from 0.038 to 0.053 per cent CO_2 (average 0.046 per cent). He found ocean air to average 0.053 per cent in the day and 0.035 per cent at night, presumably due to absorption of CO_2 by the water when cooled at night.

Since the sea contains more than thirty times as much CO_2 as the air, it must regulate the average concentration of this gas in the air. Krogh supposes that CO_2 is being increased in the air by the combustion of coal and hence the CO_2 tension of the air is greater than that of the sea. He calculates the carbon in the air as 600 billion tons, and since the annual consumption of coal is now more than a billion tons, the increase might become measurable if it were not absorbed by the sea. Chamberlin supposes that 440 million tons of carbon are withdrawn annually from the air by the weathering of rocks.

If the CO_2 content of the Gulf Stream water remains approximately constant as it flows northward, its CO_2 tension must decrease, owing to the increase in the absorption coefficient for CO_2 and the decrease in the dissociation of bicarbonates with fall in temperature. But if equilibrium with the air is partially reached, the CO_2 content and H^+ concentration should be greater in the North, which seems to be the case. Palitash found the P_{H} in the Atlantic equal to 8.1 in the far North, and 8.25 nearer the equator. If the sea locally affects the CO_2 content of the air, we might expect northern air to be poor in CO_2 , but Krogh found from 0.025 to 0.07 per cent in the air of Greenland, whereas the average for the world is taken at 0.03 per cent. Benedict found, however, from 0.01 to 0.034 per cent between Boston and Genoa, and 0.003 to 0.027 per cent between Montreal and Liverpool, with the same apparatus. It would be easier to determine whether CO_2 is being absorbed or given out by the sea by determining the CO_2 tension of the sea and air simultaneously at the same place, as I hope to do next year.

The sea water is so complex a mixture that it would be difficult to apply the law of mass action to it, but owing to the remarkable constancy in the relative amounts of the chief neutral salts in it, we may say that the P_{H} depends on the excess of non-volatile base over acid and also on the CO_2 tension, at a given temperature. This excess of base was found by Dole to be 0.00237 to 0.00257 N, but this determination seemed of sufficient importance to see whether the same result is obtained by other methods of titration as follows: The titrations were made with 0.1 N HCl. The sea water (100 cc.) was placed in a wide-mouth bottle covered by a paraffined cardboard, perforated in three places for the introduction of the burette tip, the hydrogen electrode used as

an indicator, and a rubber tube of 1 mm. bore filled with saturated KCl solution and closed at the end with a wooden plug, for connection with the calomel electrode. After dropping in a small quantity of acid a vigorous stream of hydrogen was passed through the electrode into the sea water, which was allowed to fill the electrode and was blown out with hydrogen three times; it was then allowed to rise only far enough to touch the platinized disk, and the reading was taken. This process was repeated, and the curve of P_H and cc. of acid was plotted. The angle of the curve was taken to denote the acid necessary to neutralize the excess of base over non-volatile acid. I found the excess base in sea water to be 0.0023 to 0.0025 N. Titrations with 0.05 N H_2SO_4 gave the same results, but the angle seemed not to be as sharp as with HCl. That the excess base had been neutralized at the angle in these titration curves was confirmed by adding the indicated amount of acid to 100 cc. of the same sample of sea water and determining the P_H after expelling the CO_2 more or less completely. After bubbling air through for 12 hours, the P_H was about the same as that of an NaCl solution that had been exposed to the air. After boiling it was nearly neutral, and after boiling down to half volume it was also about neutral, and the same was true after bubbling hydrogen through it for a long time. In no case was it alkaline. Artificial sea water made of absolutely neutral salts plus 24 cc. of 0.1 N $NaHCO_3$ to the liter reached a P_H of 8.2 after aeration for about 6 hours, whereas if only 23 cc. were used the aeration could be continued for a slightly longer period without danger of making it too alkaline. Since artificial sea water containing only 23 cc. of the soda solution reacted to aeration more nearly like natural sea water, this amount of soda (added as bicarbonate) is recommended in making artificial sea water, which should be aerated sufficiently to bring the P_H to about 8.15.

Although Herbst maintained the life of sea urchin eggs for some time in artificial sea water, and the experiments of many investigators have shown that the exact proportions of the salts are immaterial in many physiological experiments, Dr. A. G. Mayer informed me that no artificial sea water had been found that would maintain the jellyfish, *Cassiopeia*, in a normal condition. It pulsates intermittently (after the excitement has passed)

when put into artificial sea water and continues to do so until removed or until death ensues. It seemed important, therefore, to attempt to make a more successful sea water in order to determine, if possible, the full physiological significance of this medium. The principal salts are so well known that errors in their concentration would not be looked for in locating the trouble. The minor constituents are probably more variable, and the older analyses not so reliable, but some more accurate analyses have been made by Raben, Matthews, and others. It is possible that organisms may suffer from lack of some of these minor constituents, and it is also possible that organisms may suffer from an excess of them when they occur as impurities in the principal salts or in the distilled water. These constituents are supposed to occur as follows, in parts per million:²

NH ₃	0.0085-0.15	Cu.....	0.012(?)
Li.....	Trace.	Ag.....	0.01-0.169
Rb.....	11-15(?)	Au.....	0.005-0.065
Cs.....	Trace.	Ra.....	0.000000017(?)
Sr.....	"	F.....	0.3-0.8
Ba.....	"	I.....	0-2.19
Mn.....	"	NO ₃ +NO ₂	0.18-1.1
Zn.....	0.002	PO ₄	0.2-2.2
Fe.....	0.9-3.0	SiO ₂	0.2-1.4
Co.....	Trace	Al ₂ O ₃	0.2(?)
Ni.....	"	B.....	Trace.
Pb.....	0.1(?)	As.....	0.01-0.08

If the purest reagents are used for the principal salts in making sea water, the mixture would be supposed to contain, in parts per million, about: 0.03 Al₂O₃; 0.3 SiO₂; 0.2 Fe; 0.05 I; traces of Ba and NH₃; traces of As and heavy metals; 0.001 NO₃; and 0.0003 PO₄. Warburg found enough Cu in reagents that had been crystallized in copper dishes to increase the oxidation greatly in sea urchin eggs. Warburg found further that the addition to sea water of 0.001 Ag or 0.002 Au or 0.0006 Cu (parts per million) increased oxidation in unfertilized sea urchin eggs about 600 per cent.

² For references to the literature on this subject see the works of Clarke, Krümmel, Raben, Matthews, Roth, Murray and Hjort, Forchhammer, and Dittmar, quoted in the list of references at the end of this paper.

In order to test whether there might be enough heavy metals in "reagent," "analyzed," and "*für Analyse*" salts to affect organisms, sea water was made from them and compared with that made from further portions of the same salts after solution in water redistilled in quartz (since Locke found Cu in distilled water in a toxic concentration) and recrystallization in "pyrex" glass. The NaCl was precipitated by admitting HCl gas into its saturated solution. Instead of NaHCO₃, recrystallized Na₂CO₃ plus an excess of CO₂ was used. The results were equally favorable with the two sea waters. The addition of as much as five parts per million of PO₄ was detrimental to animals.

Since H₂O is the chief constituent of sea water, particular attention was paid to it. It was found that conductivity water made in Hulett's laboratory and sealed in pyrex flasks by fusing the glass accomplished no better results than Merck's distilled water. Pyrex glass contains arsenic, but very little should have dissolved in the water since the glass is extremely insoluble. It is said to contain no lead. The only positive result from experiments with different qualities of distilled water was that aeration improves it. The aeration necessary to bring the artificial sea water to the required P_g is not sufficient from other standpoints. It was not determined whether the beneficial effect of aeration was the addition of some element (O₂, for instance), or the elimination of gaseous impurities. Merck's distilled water was free from NH₃ but had a taste that was not lessened by redistillation in quartz. The conductivity water had this taste in a much smaller intensity, and it required 72 hours of vigorous aeration to reduce the taste (of a liter) of Merck's water to equal that of conductivity water. Since the conductivity water had purposely been kept from the air as much as possible, its lack of taste may have been due to the oxidation of all organic impurities. If this was so, the taste of Merck's water must have been due to some volatile substance other than NH₃ or CO₂.

In making the artificial sea water, the salts were made up in normal solutions (0.5 M of bivalent salts), and the following number of cc. were used to make a liter, according to an analysis of Tortugas sea water of salinity 35.49 (Clarke) or 35.41 (Dole). The proportions for isotonic solutions are also given. The

isotonic solution of NaHCO_3 is approximately isotonic after aeration, but it should be noted that these solutions cannot be exactly isotonic since mixing them affects the dissociations.

	Normal solutions.		Isotonic solutions.	
		cc.		cc.
CaCl_2	0.5 M	22.0	0.38 M	29.0
MgCl_2	0.5 M	50.21	0.37 M	67.9
MgSO_4	0.5 M	57.09	0.975 M	29.5
KCl	M	10.23	0.577 M	17.7
NaCl	M	483.65	0.568 M	852.0
NaBr	M	0.8	0.565 M	1.4
NaHCO_3	M	2.32	0.930 M	2.5
H_2O		373.63		
		1,000.00		1,000.0

The mixture should be aerated until it has a P_H of about 8.15. This artificial sea water was tested with all the delicate marine organisms available. The lagoon jellyfish, *Cassiopeia xamachana*, lived in it indefinitely and its pulsations were normal. Some died in 6 weeks if the water was allowed to evaporate, but the prevention of evaporation or the occasional addition of distilled water to restore the volume was all that was necessary to keep them alive (without food). Eggs of the Atlantic Palolo worm, *Eunice fucata*, developed in it and produced two pairs of setæ. Some were alive at an age of 13 days, although they had never been fed.

Many animals apparently live at a great depth in the sea where the water is cold, and when brought to the surface in warm regions die sooner or later, and very rapidly when brought into laboratories. Since these animals are considered to be the most delicate, I placed them in the artificial sea water. *Sagitta* lived 7 days, various pelagic medusæ, ctenophores, and siphonophores lived about 12 hours when crowded in small vessels, and *Salpa* only a few hours under the same conditions. If the P_H was changed beyond the limits, 6 to 8.26, death of all of the animals occurred sooner.

The P_H of sea water is rapidly changed by the presence of animals or eggs, even in uncovered dishes. For this reason it seemed

of interest to determine the P_H of the fluids inside invertebrates, which do not differ markedly from sea water in the salts they contain, and are often very poor in proteins. The P_H of the body fluid of the sea urchin, *Toxopneustes* (*Lytechinus*) *variegatus*, was found to vary from about 7.7 to 7.8. The blood of the conch, *Strombus gigas*, was about 7.5.

Some salts may be considered nutritive, especially to plants. The growth of marine Diatoms, but not algæ, was favored when I added 2 cc. of M $Ca(NO_3)_2$ per liter. But most of the salts, or more correctly, ions in sea water, are to be considered protective rather than nutritive, especially to animals. Since the work of Ringer, O. Loew, Loeb, Mayer, and others, the idea that one ion protects the organism from the toxic action of another ion has constantly gained ground. I have shown that certain ions increase the permeability of sea urchin and fish eggs (McClendon, 1910, 1914; McClendon and Mitchell). Since these ions are present in sea water they must be antagonized by other ions in sea water. This work was greatly extended by Osterhout who showed that certain ions increase the permeability of plant cells and others inhibit their action. If the action of all ions is of this nature there must be only two classes of ions, those that increase permeability and those that inhibit this change. The work of Ringer, Mines, and others shows the antagonistic action of ions on the heart. In order to test this hypothesis of the two classes of ions the action of ions of sea water on the pulsations of *Cassiopeia* and the heart of the conch was studied. I had found the freezing point of a sample of Tortugas sea water in 1910 to be -2.03° when corrected for undercooling, which is the freezing point calculated from a salinity of 37, whereas that of salinity 35.41 is -1.937° .³ Isotonic solutions were made as follows:

$\Delta = 1.937^\circ$: 0.565 N $NaCl$, 0.577 N KCl , 0.378 M $CaCl_2$, 0.364 M $MgCl_2$

$\Delta = 2.03^\circ$: 0.59 N $NaCl$, 0.6 N KCl , 0.395 M $CaCl_2$, 0.38 M $MgCl_2$

Whole *Cassiopeias* when paralyzed by pure $MgCl_2$ solution begin to beat after the addition of a little KCl or more $NaCl$, but not by $CaCl_2$. When paralyzed by a pure $CaCl_2$ solution they may begin to beat if sufficient $NaCl$ or KCl is added before this toxic

³ Krümmel, page 241.

solution has had time to injure the animal severely. This action of Na is inhibited by an increase in the concentration of H^+ . The rate of pulsation is above normal in pure NaCl solution, but is reduced (finally to zero) by increase in the H^+ concentration. If the P_H of sea water is changed beyond the limits, 7.5 to 8.25, the pulsation rate is finally reduced, but a long period may intervene before the pulsations respond unless the change in P_H is great.

The pulsation rate is increased by the addition of KCl to sea water, and its action is inhibited by increase in H^+ . If the *Cassiopeia* is paralyzed by a marked increase in the H^+ concentration of sea water it may beat after the addition of KCl. When paralyzed by $MgCl_2$ or $CaCl_2$ it may be caused to beat by increasing the OH^- concentration.

The heart of the conch will beat if perfused with sea water. In these experiments the auricular wall was used to make connection with a glass reservoir, and a hydrostatic pressure of about 2 cm. of water was substituted for the auricular action; hence only the ventricular action was studied. It stops in systole if the P_H of the sea water is changed to 9.7, and in diastole if the P_H is 5.6. Sea water of $P_H = 8.26$ deposits $CaCO_3$ on glass, and a precipitate occurs throughout the solution if the alkalinity is increased much further, but it may remain in a supersaturated condition, as regards $CaCO_3$ for some time. It is improbable that a precipitation of $CaCO_3$ occurs in the tissue before it appears generally, since the tissue is constantly reducing the alkalinity by the production of CO_2 . Within the limit of $P_H = 9.5$, an increase of OH^- merely increases the rate of pulsation. The effect of the ions may be summarized: Increase in concentration of OH^- , Na, or K increases the rate and finally stops the heart in systole; whereas H^+ , Mg, and Ca decrease the rate and may stop the ventricle in diastole. This action of Ca is only seen when it is applied suddenly in great excess, since the discovery of Ringer that Ca^{++} favors systolic contractions holds true for the conch ventricle. When placed in equal parts of sea water and isotonic $CaCl_2$ solution, the ventricle stops in diastole but soon shrinks up and cannot be revived. In the following tabulation, the numbers express the volumes of neutralized sea water (n. s. w.) to which one volume of isotonic chloride solution is

added, and the effect produced, followed by the effect of the addition of another solution.

4 n. s. w. + Na increases rate, + HCl to make 0.001 N, stops in diastole.
 11 " + K " " , + HCl " " 0.001 N, " " "
 4 " + Mg stops in diastole, + OH⁻, starts again.
 1 " + Ca " " " then passes into rigor.

We may sum up these experiments on *Cassiopeia* and the conch ventricle in the light of electrical conductivity experiments on animals and plants by assuming that Na⁺, K⁺, and OH⁻ increase the permeability of the plasma membrane and that Ca⁺⁺, Mg⁺⁺, and H⁺ inhibit their action, thus causing decrease in permeability when the antagonistic ions are present. It has been shown that muscular contraction is accompanied by increase in permeability (McClendon, 1912) which may explain the fact that Na⁺, K⁺, and OH⁻ favor systole, and Mg⁺⁺ and H⁺ favor diastole, but the experiment must be performed in a certain way in order to show that Ca⁺⁺ may produce diastole. The anomaly that a small amount of Ca⁺⁺ favors the systolic contractions produced by a large excess of Na⁺ may be explained by the assumption that the optimum ratio of each pair of antagonistic ions is different for each part of an organism and that more than one of these hypothetical parts are concerned in any one of the experiments. For instance, suppose the optimum ratios are as follows, Ca: Na = 1 : 100 for muscle, 1 : 50 for nerve fiber, 1 : 25 for motor end plate, 1 : 10 for nerve cell body, it is clear that no ratio of Ca to Na would be especially favorable for an action in which all of these structures take part. In sea water we have a summation of antagonisms in which Na⁺, K⁺, and OH⁻ are combined against Ca⁺⁺, Mg⁺⁺, and H⁺, and the proof that it is favorable for all of these structures is that it works. It should be added that some of the minor constituents of sea water take part in these antagonisms, and that their effect is large in comparison to their concentration.

Since the statement was made by Mines that the heart of a species of *Pecten* would not beat in sea water unless it is neutralized or slightly acidified, it seems worth noting that this is not general even for molluscs of this particular group. The heart of *Pectenella* will beat in normal, neutral, acid, or hyperalkaline sea water, and even in hyperalkaline NaCl solution.

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15

**IMPROVED GAS CHAIN METHODS OF DETER-
MINING HYDROGEN ION CONCENTRATION
IN BLOOD**

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MINNESOTA, MINNEAPOLIS)**

**REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXIV, No. 4, APRIL, 1916**

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(Received for publication, December 1, 1915.)

The Gas Chain Method for Blood.

The most accurate determinations of the hydrogen ion concentration of human blood seem to be those of Hasselbalch on defibrinated reduced blood resaturated with CO₂ at alveolar tension. The objections to his method are first, the difficulties of manipulation; second, the time required to reduce the blood; and third, the error due to the deterioration of electrode and blood during reduction.¹

In order to avoid loss of CO₂ and of time in the reduction of blood, I devised an electrode that could be filled directly from the blood vessel.² But in order to determine the buffer value of blood and to avoid the use of the expensive hirudin, I have modified it as follows.



FIG. 1. Hollow needle, 7 cm. long and 0.7 mm. bore.

The blood is drawn from the vein by means of the hollow needle (Fig. 1), of 0.7 mm. bore and 7 cm. long, connected with the 12 cc. defibrinating tube (Fig. 2) by means of a short piece of strong rubber tubing, in such a manner that all of the blood that is exposed to the air overflows out of the tube. A piece of glass tubing is connected to the open end of the defibrinating tube by means of rubber tubing. When the defibrinating tube is full the two rubber tubes are closed with pinch-cocks or Langenbeck

¹ Hasselbalch, K. A., and Gammeltoft, S. A., *Biochem. Z.*, 1915, lxxviii, 235, 236.

² McClendon, J. F., *Am. J. Physiol.*, 1915, xxxviii, 181.

clips and the blood is defibrinated with the lead ball by shaking the tube. As the blood cools and contracts the clip is opened from time to time in order to allow blood to return from the overflow tube and thus prevent the escape of gas from the blood, due to diminished pressure.



FIG. 2. 12 cc. defibrinating tube.

When defibrination is complete the closure of the rubber tubes is secured by folding them back over the nipples of the defibrinating tube and wrapping them with wire or cord. The defibrinating tube is then placed in the centrifuge, balanced with a similar tube, and the fibrin and corpuscles are precipitated. When it is removed from the centrifuge the wires are replaced by clips. The lower rubber tube is connected to a long rubber tube and funnel filled with mercury in such a manner that no air is admitted. The upper rubber tube is connected with the left end of the electrode of 5 cc. capacity shown in Fig. 3. By opening the cocks and raising the funnel, the electrode is filled with serum in such a manner that all of the serum that comes in contact with the air overflows through the stop-cock into the overflow tube.

A small bubble (0.1 cc.) of pure hydrogen (or $H_2 + CO_2$) is admitted into the electrode and both ends of the latter are closed. The electrode is shaken until the bubble comes to equilibrium with the serum as regards CO_2 tension, when the bubble is allowed to rise until it completely surrounds the platinum foil. The electrode is then set up and allowed to come to the temperature of the room. It is connected to the electrometer by means of a copper wire hook that engages a hook on the end of the platinum foil. The copper wire is long and coiled in a close spiral to reduce its stiffness, and is small enough so that its weight does not straighten out the hook on the platinum. If copper and platinum are bright and dry the contact is usually good enough to get the zero point with the electrometer, and if this is not the case it is immediately detected by failure of the electrometer to show a sharp zero point on the potentiometer wire. The overflow tube is connected with the saturated KCl solution into which the calomel electrode dips, by a piece of absorbent cord. The

stop-cock is not greased and need not be opened while taking a reading, but must be opened momentarily immediately before the reading in order to equalize the pressure of the hydrogen, which has been reduced by partial combination with oxygen dissolved in the serum.

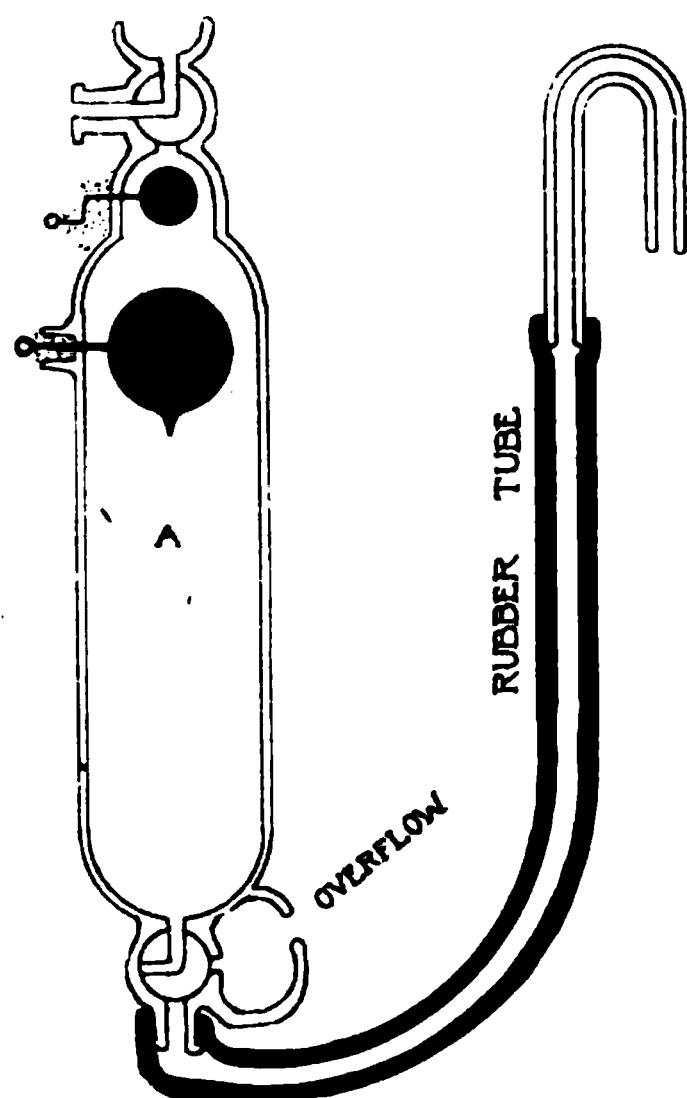


FIG. 3. Hydrogen electrode, actual size. The stop-cocks are bored at right angles so that the connecting tubes may be rinsed out through the extra openings in the stop-cocks. The lower cock is lubricated with KCl solution before filling the electrode, and its bore may be filled with the same solution and turned as in the figure if it is desired to have a wider surface of contact between this solution and the electrode contents. The two discs are gold or gold plated platinum and are platinized. In case CO_2 loss is to be avoided the upper one is used, but with known constant CO_2 pressure, the lower one is used and the H_2 — CO_2 mixture forced in until only the point of the disc touches the liquid. The rubber connection filled with KCl solution allows mechanical inversion of the electrode about the axis, A, without disconnection from the potentiometer (thus replacing the absorbent cord).

A succession of readings are taken until a maximum difference of potential is obtained and remains constant 5 minutes, which denotes that all of the oxygen contained in the hydrogen has disappeared. If it is not certain that CO_2 equilibrium was established the electrode must be shaken again, after which some time

will be required to reduce the additional oxygen that has been brought up from the lower part of the electrode. Usually 200 inversions of the electrode are sufficient for CO_2 equilibrium and 10 minutes sufficient for disappearance of the oxygen.

After the above procedure the serum has the same hydrogen ion concentration as the blood in the vein except for temperature change and the loss of CO_2 into the hydrogen. By reducing the ratio of hydrogen to serum the loss of CO_2 may be made small. It may be still further reduced by using hydrogen containing 5 per cent CO_2 .

The platinum foil should be 0.02–0.05 mm. thick, or if a wire is used, the part inside the electrode vessel should be beaten thin and thoroughly cleaned.

The electrodes are cleaned with potassium bichromate in H_2SO_4 , are replatinized for about 30 seconds, and are tested with a standard phosphate mixture. Ten freshly platinized electrodes are tested with it and unless the majority of them agree to a millivolt the determination is repeated.

The rubber tube for the defibrination apparatus must be very strong and renewed each time. It is well to attach it with rubber cement and wire before the experiment.

The Determination of the CO_2 Pressure on the Same Sample.

Having once determined the C_H of the serum, which is the same as that of the blood in the vein, the CO_2 pressure of the sample may be approximated by exposing it to a series of CO_2 pressures and noting under which the C_H is nearest to the original value.

The CO_2 and H_2O are mixed in a tonometer. For this purpose the tonometer is made like a Hempel burette, except that one end is wide and the other constricted and finely graduated from 0 to 10 cc. The tonometer is set up vertically with a mercury funnel and a 1 cm. vertical glass tube used as a sight in leveling the mercury surfaces. When the mercury in the funnel, tonometer, and 1 cm. tube is on a level the amount of gas introduced may be read on the scale. The tubes leading from the CO_2 and H_2 generators are connected with side tubes several meters long, filled with the respective gases in order to maintain them at atmospheric pressure while being introduced into the tonometer.

The serum is transferred to the tonometer and rotated 15 minutes by a rubber band that passes over it and an axle. The serum is returned to the electrode, a bubble of the same gas mixture added, and the reading taken. If care is taken in this manipulation, no oxygen is admitted and equilibrium is attained more quickly than for the first reading, sometimes in 2 minutes. The next lower per cent CO_2 is now used, and so through the series. The results may be compared with the alveolar CO_2 pressure.

Knowing the alveolar CO_2 tension, one can determine the P_a of arterial blood. We find it the same as venous blood within the limits of error of a single determination (without averaging duplicates). By making both electrodes the same size, duplicate determinations may be made in the same electrode vessel. If the volume of pure hydrogen is increased to 1 cc. the maximum error is 1 millivolt.

It is claimed by Hasselbalch that serum is more alkaline than blood. Hasselbalch admits that adding oxygen to blood makes it read less alkaline. Evidently oxygen acts by combining with the hydrogen in the platinum black. Serum reads more alkaline than blood because it contains less oxygen, but the reading is more nearly the true reaction of the blood. In order to decrease the danger of introducing oxygen the electrode is filled with hydrogen before use.

The Buffer Value of Serum.

Since the buffer value of a fluid means its resistance to change of reaction by the addition of acids or bases, the change of reaction on the addition or removal of CO_2 gives an index of the buffer value. It is only necessary to determine the C_s of the serum at two known CO_2 pressures to obtain an index of the buffer value. It would be convenient to have some standard of pressure, and 10 per cent and 3 per cent of an atmosphere are suggested. If the CO_2 pressure of the serum has been determined by the above method, these data are already at hand; if not, the rule should be always to begin with the higher pressure.

Thermoregulation.

Owing to the inconvenience of small thermostats, the laboratory was kept within 0.3° of 22° by means of an electric fan and the apparatus shown in Fig. 4. The essentials of a good system are low heat capacity of regulator and heater and rapid conduction. The regulator is made of a strip of invar and brass welded together (combined thickness, 1 mm.). The heater is bare nichrome wire strung through the air.

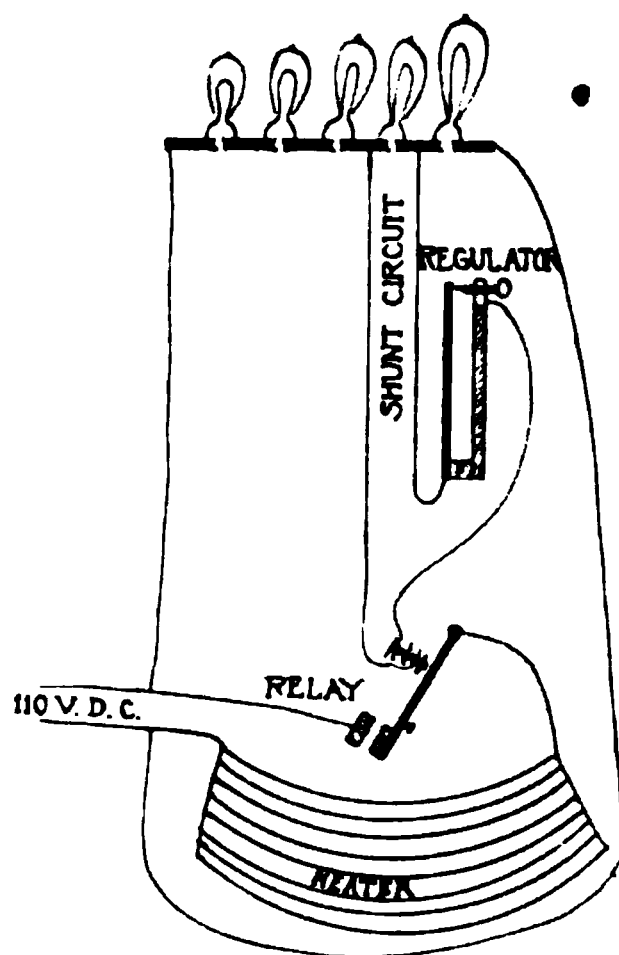


FIG. 4. Thermoregulator.

Table for Converting Millivolts to Hydrogen Ion Concentration.

Since the table by Schmidt³ is for only one temperature, 18° , the range of $18\text{--}30^\circ$ was selected in preparing the graph in Fig. 5. In 1908 the International Electrical Congress established the international ohm and fixed the ampere as the current that deposits 0.001118 gm. Ag per second. Taking the atomic weight of Ag as 107.88, the Faraday or electrical equivalent is 96,494 coulombs. The value 96,500 has come into use. If these new data are used

exclusively, the formula for C_H is $P_H = -\log C_H = \frac{\text{E. M. F.}}{0.0001984 T}$

³ Schmidt, C. L. A., *Univ. California Publications, Physiology*, 1905-10, iii, 104.

which is nearly the same as the old formula. The value of the volt was still uncertain, however, until the International Committee, at Washington, 1910, standardized the method of washing the cathode of the silver coulometer, and fixed the E.M.F. of the International Standard Weston Cell at 1.0183 at 20°. According to Rosa,⁴ it is more nearly 1.01827, but this difference is less than the individual variations of cells. It was pointed out by Wolff⁵ that no exactly correct formula for the temperature effect on the E.M.F. has been devised, and that from 2 to 24 hours are necessary for equilibrium after an abrupt change of temperature. From Wolff's formula, which is more accurate than previous formulas, it may be seen that the change in E.M.F. per degree is more nearly 0.04 millivolt than 0.038 as given by Jaeger and Wachsmuth. Where temperature control is not adequate, it might seem safer to use a cell from the Weston Company, saturated at 4°, E.M.F. = 1.0186, independent of temperature.

There is considerable difference in the correction for calomel electrodes as applied by different writers. G. N. Lewis⁶ uses a value 8 millivolts different from that used by other investigators for the normal calomel electrode. He bases this value on a calculation of the dissociation of the acid used in the normal hydrogen electrode from thermodynamical data, which makes the dissociation of strong acids more nearly obey the law of mass action. Since so much work is based on the calculation of C_H from electrical conductivity, it might seem safer at present to continue this practice.

Although the saturated KCl calomel electrode of Michaelis⁷ is the most convenient, if work is done at various temperatures the 0.1 N KCl calomel electrode is better on account of the lower temperature coefficient. Since there is a variation in the value of the correction for the 0.1 N electrode used by different writers, it is hoped that some standard value may be decided on. Ostwald⁸

⁴ Rosa, E. B., Vinal, G. W., and McDaniel, A. S., *Bull. of the Bureau of Standards, U. S. Dept. of Commerce*, 1913, ix, 493.

⁵ Wolff, F. A., *Bull. of the Bureau of Standards, U. S. Dept. of Commerce*, 1908, v, 309.

⁶ Lewis, G. N., and Randall, M., *J. Am. Chem. Soc.*, 1914, xxxvi, 1969.

⁷ Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 1914.

⁸ Ostwald, W., and Luther, R., *Physiko-chemische Messungen*, Leipsic, 3rd edition, 1910.

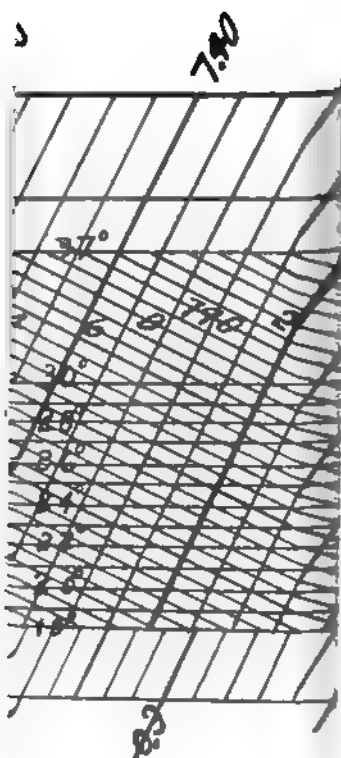
found no difference due to size of grain of calomel, since all of calomel contain grains of various sizes, and the only variation is due to impurity of ingredients or incorrect concentration of KCl, which can easily be avoided. It is well to seal the electrode in a flame and have the tip of the syphon always closed by a greased stop-cock or ground cap which is immersed in a vessel of 0.1 N KCl solution; a second syphon connects the saturated KCl. Auerbach,⁹ who corrects for the water vapor pressure of the normal hydrogen electrode, found the E.M.F. of this electrode coupled with the 0.1 N KCl calomel electrode to be 0.337 volt at temperatures from 0-30°, and it would be convenient if this value were accepted generally. Our formula then becomes

$$P_H = -\log C_H = \frac{E.M.F. - 0.337}{0.0001984 T}.$$

The partial pressure of hydrogen in the hydrogen electrode is reduced by [(760 - barometric pressure) + (mm. CO₂) + (mm. N₂) + (vapor pressure of water)]. This may be made equal to 760 mm. by applying pressure to the overflow tube of the hydrogen electrode and closing the stop-cock. The reading may be corrected by adding to it $\frac{0.0001984 T}{2}$

where q is the partial pressure of hydrogen in the electrode. The nitrogen pressure may be taken at 10 mm. and the vapor pressure is practically that of pure water. The correction for the vapor pressure is about 1.7 mv. with the barometer at 740.

⁹ Auerbach, F., *Z. Elektrochem.*, 1912, xviii, 13.



Converting millivolts into P_{50} and

AN IMPROVED HASSELBALCH HYDROGEN ELECTRODE AND A COMBINED TONOMETER AND HYDROGEN ELECTRODE, TOGETHER WITH RAPID METHODS OF DETERMINING THE BUFFER VALUE OF BLOOD.*

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Minneapolis.)

(Received for publication, May 31, 1916.)

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1. The Two Compartment Hydrogen Electrode.

After experimenting for a year with hydrogen electrodes based on the form designed by Michaelis, the senior author attempted to modify Hasselbalch's electrode so as to eliminate the danger of loss of CO_2 during transfer of solution. The electrode vessel (Fig. 1) has two compartments, the smaller of which contains the electrode proper. Hydrogen is shaken with a portion of the solution in the large compartment and then passed through the large middle stop-cock into the smaller compartment containing another portion of the solution, where the reading is made. Stop-cock *a* is not greased and conducts electrolytically while closed. Since the hydrogen is brought to an equilibrium with the first portion of the solution, it does not appreciably remove CO_2 from the second portion. The lower the buffer value of the

* The Apparatus was bought out of the Research Fund of the Graduate School.

solution, the larger the large compartment should be. If the two compartments are the same size, fairly accurate readings may be made on blood, but if the samples are not exceedingly small it is better to have the ratio of the compartments as in Fig. 1. A demonstration of the reliability of this electrode was made by charging it with blood serum and making a succession of readings from the end of 5 minutes until the end of 48 hours; they were the same within 1 millivolt. The potentiometer and Weston cell were compared with ones recently calibrated by the Bureau of Standards.

A description of the technique of determinations on blood will suggest the precautions necessary for any solution. The blood

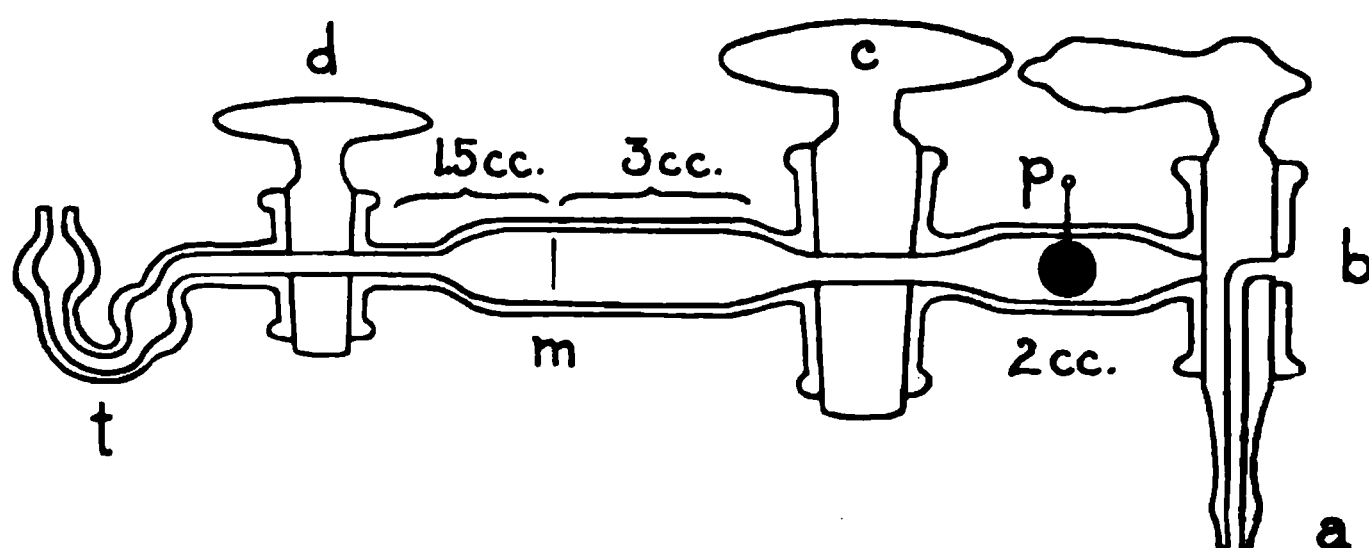


FIG. 1. Two compartment hydrogen electrode. Hydrogen is shaken with one portion of the blood in the large compartment and passed into another portion of the blood in the small compartment for the measurement of the electrode potential.

is collected as described by McClendon (1916 a), and the defibrinated blood or serum used, or it is passed directly into the electrode by means of a rubber tube connected to the needle or cannula in the blood vessel. In the latter case, the electrode must contain some hirudin or isotonic, neutralized sodium oxalate. The dilution of blood with an equal volume of a neutral solution of low buffer value has been shown by Michaelis, and more recently by Corral, to have no perceptible effect on the reaction.

In order to obtain absolute values, the electrode must be cleaned with potassium bichromate in H_2SO_4 and replatinized a few seconds by removing the stop-cock *a* and inserting a platinum wire anode through the opening *b* (Fig. 1). It is then thor-

oroughly rinsed with distilled water (and the stop-cock *a* lubricated with serum or KCl and glycerol) and filled with pure hydrogen; a drop of water in the trap *t* serving to prevent the backward diffusion of air until all stop-cocks are closed. The rubber tube admitting the blood is connected to *a*, and the air in it allowed to pass out at *b*, after which this stop-cock is turned so as to admit the blood into the electrode, when the other stop-cocks are opened. The blood is allowed to enter until it reaches the mark *m*, the apparatus then containing 5 cc. of blood and 1.5 cc. of hydrogen.

All the stop-cocks are closed and the apparatus is shaken or inverted 200 times. The middle stop-cock is opened and the apparatus tapped or swung so as to cause the hydrogen to pass into the smaller compartment by displacing part of the blood. This backflowing blood helps to complete the equilibration of the hydrogen in regard to CO_2 . The smaller compartment now contains 1.5 cc. of hydrogen and 0.5 cc. of blood, and is shaken or inverted 200 times; and the stop-cock at *a* is immersed in the KCl solution connected with the calomel electrode. The connection is completed by hooking a wire in the platinum loop *p*, the wire being bright and weighted sufficiently to maintain a good contact.

If there is but little oxygen in the blood, the definitive reading should be obtained immediately (provided it is the same temperature as the calomel electrode), but if it contains much oxyhemoglobin the reading will be too low, and will slowly rise as the oxygen combines with the hydrogen in the platinum black. If more than 15 minutes are allowed to elapse, the electrode should be shaken in order to bring a fresh layer of blood into contact with the platinum before another reading. It is not necessary to shake continuously, as recommended by Hasselbalch, but continuous shaking hastens the disappearance of the oxygen. If much hydrogen disappears by combination with the oxygen, it is necessary to restore atmospheric pressure by momentarily opening the stop-cocks *c* and *d*, but this cannot be done more than once without danger of admitting oxygen from the air.

Since convenience and accuracy depend on the details of the construction of the electrode, a few words as to its manufacture may be permitted. If platinum is used for the electrode proper,

it must be exceedingly thin so as not to absorb an appreciable amount of hydrogen. Gold is much better, but must be attached to a platinum wire fused through the glass. Platinum may be welded to platinum at a white heat, but gold melts at this temperature. It is better to melt a drop of gold on the end of the platinum wire and hammer this drop (after cooling) to the gold disc. In doing this, a gold wire or scrap of foil is wrapped around the end of the platinum wire and held in the flame until it is just melted. If held in the flame longer, the gold will amalgamate with the platinum, forming a brittle alloy.

The bore of stop-cock *c* should be at least 3 mm., as the larger it is the more easily the hydrogen is caused to pass into the small compartment.¹

2. The Hydrogen Ion Concentration of Blood and Serum.

It is stated by Hasselbalch and others that serum is more alkaline than blood. Hasselbalch found that the less oxygen blood contained, the more alkaline it appeared to be when measured by the hydrogen electrode. Since hemoglobin may carry a large store of oxygen, the discrepancy between the reaction of blood and serum is apparently due to faulty technique. Although it is theoretically impossible for the mere proximity of the corpuscles to change the reaction of the plasma, and Michaelis claims that coagulation does not affect the reaction, it seemed advisable to test this question experimentally. The result was that the reaction of serum and defibrinated blood from the same source and without loss of CO₂ is the same. This tells nothing concerning the reaction of the interior of the corpuscles or the effect of laking on the reaction of the blood, which will be considered in a later paper.

Hasselbalch states that the P_H ($= -\log H^+$) of blood is 0.17 lower at 37° than at 19°, and Michaelis states that P_H determinations at 37° are 0.21 lower than the average of determinations on different persons at room temperature. Since Corral and others have not observed high temperature coefficients for the hydrogen

¹ The best electrodes were blown by A. S. Jones, 62 Alexander St., Princeton, N. J., who also made the stop-cocks, and the tonometers to be described.

ion concentration of various buffer mixtures, it seemed strange that the blood should be an exception. In order to test this, two saturated KCl calomel electrodes were compared at the same temperature and found to be alike. One of them was placed in a thermostat at 37° and allowed to remain 3 days, since Wolff found it took a long time to come to equilibrium. Blood and serum were tested at 23° and at 37° against these calomel electrodes. The difference in the P_H at the two temperatures was not greater than 0.02 and was therefore within the limit of experimental error. The same was found true of NaHCO_3 solutions. This experiment was repeated with 0.1 N KCl calomel electrodes. The P_H was lower at the higher temperature, but the difference did not exceed 0.01 to 0.07 when corrected for the increased vapor tension of water.

The reason for using two types of calomel electrodes is that the result depends on the value of the E. M. F. of the calomel electrode against the normal hydrogen electrode, as calculated by different investigators. In such calculations the hydrogen ion concentration in the hydrogen electrode is determined by electric conductivity or other indirect means. Ellis observed that the H ion concentration of HCl, even at 0.005 N, is different, when calculated from conductivity data, from the value obtained by electrode potential. For this reason the results obtained on blood at different temperatures are not to be considered absolute, but merely reproducible by using the ordinarily accepted data, as given by McClendon (1916 a, Fig. 5) and Michaelis.

Since the viscosity of water decreases with rise of temperature the dissociation of acids, calculated from conductivity data, is erroneous unless these data are corrected for viscosity. Owing to the increased dissociation at higher temperature, the P_H of pure water is about 0.3 less at 37° than at 18°, but the P_H of an alkaline solution should be greater at the higher temperature. We found the P_H of N NaHCO_3 to be 7.9, the P_H of 0.1 N solution, which is dissociated (hydrolytically) to a greater extent, to be 8.3. The latter result confirms that of Auerbach and Pick. Apparently the effect of rise in temperature increasing the dissociation of water and thus decreasing the P_H is partly counterbalanced by the greater dissociation of alkaline salts, increasing the P_H , so that the reaction of the blood remains nearly the same when

measured by means of the hydrogen electrode at different temperatures. The dissociation of water and hence the hydroxyl ion concentration in the blood increase with rise in temperature; hence the blood becomes more alkaline with rise in temperature, as pointed out by Höber.

Hasselbalch has shown that the P_H of arterial blood (*i.e.*, at alveolar CO_2 tension) is remarkably constant. On the contrary, Menten and Crile claim that the blood returning from different organs shows great differences in P_H . The unpublished determinations which the senior author made for Dr. Uhlrich, using electrodes previously described, showed variations in the P_H of venous blood. The average P_H of venous blood is less than 0.05 lower than that of arterial blood, and smaller fluctuations in the CO_2 content must necessarily have less effect on the reaction. The records in the literature of the CO_2 content of blood returning from various organs are very discordant. According to Hill and Nabarro, activity of an organ sometimes reduces the CO_2 content of its venous blood because the effect of increased metabolism is overcompensated by vascular dilatation. Perhaps the chief cause in the variations in the P_H of venous blood is the variation in the blood flow, and hence stasis attending the collection, which is more or less unavoidable in the human subject, is a real source of error.

It has been thoroughly established that changes in the H ion concentration of the blood affect the respiratory center, but for those who hope to detect a change in the reaction of the blood during dyspnea, a little calculation may be of interest. Haldane and Priestley observed that a rise of 0.2 per cent in the CO_2 of the alveolar air doubles lung ventilation. The change in the H ion concentration of the blood caused by a rise in the CO_2 tension of 0.2 per cent of an atmosphere is far within the limits of error of the gas chain method as applied to blood. Hence measurable changes in the P_H of arterial blood mean changes in the threshold for stimulation of the respiratory center as in shock or after drugs.

The respiratory center is apparently not so sensitive to the infusion of HCl into the blood, but this may be due to a difference in permeability and concentration gradient. Since CO_2 is produced by the respiratory center, an increase in this gas in the blood

would decrease the outward diffusion from the center, which would become less alkaline. A mere increase in the H ion concentration of the blood would have much less effect on the diffusion of CO_2 , since only a minute fraction of the latter is dissociated. If acid were injected until free acid appeared in the blood, some would diffuse into the respiratory center. Judging by experiments on other cells, fatty acids should diffuse in faster than mineral acids, and hence be more effective in increasing respiration, as is actually found to be the case.

When HCl is added to blood *in vitro*, its content of free CO_2 is increased, but it is incorrect to suppose that the same final result is obtained by infusion of HCl. In the latter case the respiratory center is stimulated and the CO_2 eliminated, so that in a short time the CO_2 tension of the blood is below normal. Since the content of the blood in free CO_2 varies directly with the CO_2 tension, the infusion of HCl reduces the free CO_2 in the blood. The reduction is not sufficient, however, to maintain the normal reaction of the blood, as first shown by Szili.

Owing to the impossibility of measuring the H ion concentration of the respiratory center itself, all factors with a possible influence on it should be studied. For this reason attention is called, in the next section, to the buffer value.

The normal P_{H} of venous blood seems to fluctuate about 7.5, and that of arterial blood to be a little higher, but the difference is not usually measurable. The number of determinations have been too few, however, to separate individual variations entirely from errors, and no absolute values can be given.

3. The Combined Tonometer and Hydrogen Electrode.

A combined tonometer and hydrogen electrode was described by Peters, but the large rubber stopper rendered it objectionable. The form shown in Fig. 2 has been found convenient and reliable. The gold disc with platinum wire *p* is sealed in the 2 cc. compartment, and the stop-cock *c* is lubricated with a conducting solution and immersed in the KCl trough so that this part of the apparatus can be used as a hydrogen electrode in the same manner as the form shown in Fig 1. The description of the manipulation of the apparatus for blood is the same as for any other fluid. The blood is admitted at *a* until it completely fills the

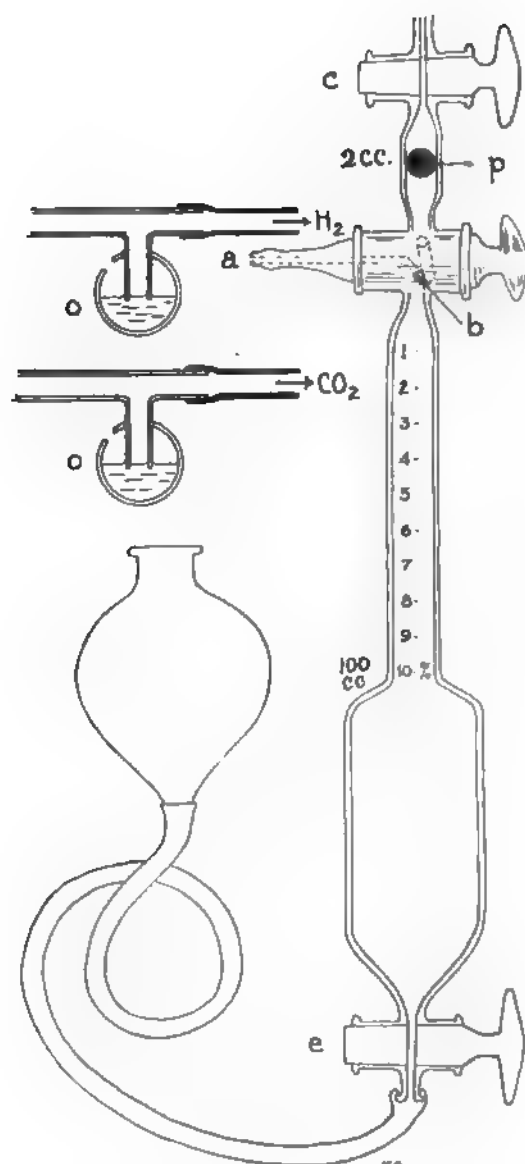


FIG. 2. Combined tonometer and hydrogen electrode. Blood is passed from the 2 cc. compartment into the 100 cc. tonometer and back into the 2 cc. compartment, where the electrode potential is determined.

2 cc. compartment, and the stop-cocks are closed. The apparatus is set up vertically and a rubber tube from a mercury funnel attached to the lower end *e*. By raising the funnel, the 100 cc. tonometer is filled with mercury which forces the air out at *a*. The tubes from the hydrogen and CO₂ generators are provided with overflows *d* to keep these gases at constant and approximately atmospheric pressure. The CO₂ is admitted at *a*, and the stop-cock *a* turned so as to force out the contained air at the side opening *b*, and then turned back so as to allow the CO₂ to enter the tonometer when the funnel is lowered. When the required per cent of CO₂ is read off on the graduations of the tonometer, the stop-cocks *e* and *a* are closed, the hydrogen tube is connected to *a*, the air washed out at *b*, the tonometer filled with this gas, and *e* closed. The stop-cock *a* is so turned as to connect the 2 cc. chamber with the tonometer by means of a hole that is at least of 3 mm. bore. By tapping or swinging the apparatus, the blood is shaken down into the tonometer, which is rotated on its long axis (placed horizontally) by means of a rubber band passed around a revolving axle above it. About 0.5 cc. of the blood is shaken back into the 2 cc. chamber, *c* immersed in the KCl solution, *p* connected to a wire, and the reading taken in the usual manner.

The remainder of the blood in the tonometer is forced into the 2 cc. chamber by means of mercury, and the second CO₂ mixture made in the tonometer in the same manner as the first. The same sample of blood will do for a series of determinations provided the mercury is pure. Before using the apparatus, it should be prepared in the same manner as the electrode shown in Fig. 1.

4. Charts for Finding the Buffer Value of Blood and Serum.

In a previous paper (McClendon, 1916 a) the buffer value was indicated by the difference between the P_{H} at 3 per cent and at 10 per cent CO₂ tension. The difference in this index of the buffer value for different bloods is so small as to make the charts shown in Fig. 3 desirable as aids in its determination.* They will at

* The quantity of data used in the making of these charts is not as large as might be desired, but the fact that the junior author cannot continue the work makes it necessary to publish them in their present form.

least serve as guides in the construction of similar charts by investigators engaged in similar work. The data on blood are not in disagreement with the limited observations of Hasselbalch, but no comparative data on serum were found in the literature. The P_H values found by Höber are too low.

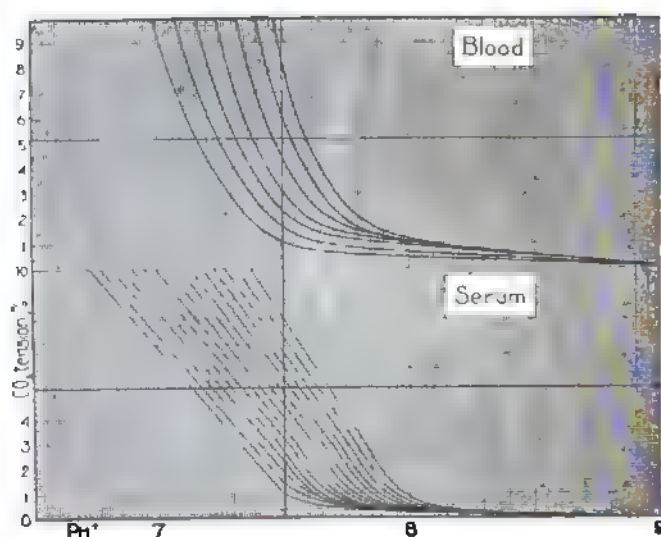


FIG. 3. Curves showing the change of P_H ($= -\log H$ ion concentration) with change of CO_2 tension. On the ordinates are measured the percentages of an atmosphere in moist CO_2 , and these may be reduced to mm. of dry CO_2 by multiplying by 7.2, since the tension of water vapor was 20 mm. and the barometric pressure 740 mm. At high altitudes all calculations should be made in mm., but near the sea level the error is not appreciable if the calculations are made in percentage of an atmosphere, as a slight change in CO_2 tension makes a much smaller change in P_H . The electrode potential calculated for 760 mm. of dry hydrogen was used in determining the P_H .

Since the P_H of arterial blood both in health and disease is remarkably constant (about 7.5) the curve in the chart corresponding to any sample of blood or serum may be found by one observation. If the alveolar CO_2 tension by the Haldane method is determined, the curve is the one passing through the intersection of this CO_2 tension and the $P_H = 7.5$ line. If the P_H at any known CO_2 tension is determined, the curve is the one

at the intersection of these P_{H} and CO_2 tension coordinates, and the intersection of this curve with the $P_{\text{H}} = 7.5$ line shows the alveolar CO_2 tension (unless the respiratory center is abnormal as in shock or after caffeine or morphine).

The alveolar CO_2 tension by Haldane's method is practically the same as that of the arterial blood. The alveolar CO_2 tension by Plesch's method may, by proper manipulation, be made to show the average CO_2 tension of the venous blood. The normal arterial CO_2 tension of the arterial blood is about 5.2 per cent, and of the average venous blood about 6 per cent of an atmosphere, or about 16 per cent higher than arterial. Higgins used a modified Plesch apparatus, in which the entire air was rebreathed four times (about 20 seconds), and obtained values about 20 per cent higher than by Haldane's method. It would be interesting to know whether such a ratio is constant even when cases of extremely low CO_2 tension are included. If such were the case, the true alveolar CO_2 tension might be calculated from the results by the Plesch method.

The striking fact shown by the charts is that the buffer value of the blood or serum depends on its CO_2 tension. Below 1 per cent CO_2 tension, the buffer value is almost nil. Above 5 per cent CO_2 tension, the buffer value of the blood is almost infinite, while that of the serum is somewhat less. Therefore, the buffer value of the blood of a patient with alveolar CO_2 tension equal to less than 1 per cent of an atmosphere, is so low as to make it probable that great local differences in the reaction of the blood exist in the body. Such local variations probably have a great deal to do with the dyspnea of many patients. The acid produced in the respiratory center itself may be very poorly neutralized by the blood, and temporary increased breathing must result.

6. The Indicator Method for Determining the Buffer Value.

At the request of Dr. Rowntree we have calibrated his indicator method for determining the reaction of blood. Phosphates of known water content were used to make solutions according to Sørensen's directions, and portions of the same phosphates sent to Hynson, Westcott and Company for the manufacture of

sealed tubes of standard solutions colored with phenolsulfonephthalein. These tubes were then compared with similar ones calibrated by the gas chain method. The only difficulty experienced was in obtaining "nonsol" tubes of exactly 1 cm. bore, a small divergence from which caused an appreciable error. Blood was tested by the gas chain method and then dialyzed 7 minutes against isotonic, neutral (tested) NaCl solution in *stoppered* "nonsol" tubes and tested with phenolsulfonephthalein. If the transfer and other manipulations were sufficiently rapid so as not to lose CO₂ appreciably into the air, and the tubes were of exactly 1 cm. bore, the results did not disagree with those of the gas chain method. The latter could be read, however, to one more decimal place. In attempting to prevent CO₂ loss by a layer of oil, it should be remembered that CO₂ is more soluble in oil than in water, and the oil merely lessens convection (by its non-miscibility).

The following method for determining the buffer value clinically is suggested. 3 or more cc. of blood mixed with hirudin or neutralized oxalate are introduced into a 100 cc. tube with openings at each end of 1 cm. bore provided with rubber tubes and pinch-cocks. The blood is introduced into this tube and the breath of a person of normal (previously tested) alveolar CO₂ tension blown through it, the pinch-cocks being closed after the last breath is forcibly expelled. The tube is rotated 10 minutes and the reaction of the blood determined by the indicator-dialysis method. The intersection of the line of this P_H with the 5.2 per cent CO₂ tension line in the chart will show the curve corresponding to the blood sample. The intersection of this curve with the 7.5 P_H line will show the alveolar CO₂ tension of the patient (the error will be small except in case of shock or after certain drugs).

6. *The Indicator Method for P_H of Stomach Contents.*

Stomach contents were dialyzed through collodion sacs as first tried by Fowler, Bergeim, and Hawk. The buffer value varies directly with the P_H and is very low for high acidity, in which case the volume of the stomach contents in the sac should far exceed that of the 1 per cent NaCl solution in the tube containing the sac. For low acidity, Davidsohn has shown that dilution affects the P_H very little.

The dialysate was compared with the published colored chart (McClendon, 1916 b), using the indicators noted in the chart. A calibration of this method showed that it was significant for the integral part of the P_{H} . Sealed tubes of standard solutions with some of the indicators were made also but, unfortunately, methyl violet, the most useful indicator, fades in very acid solutions. Mauveine was not obtainable, but it is hoped that it would not fade so rapidly. Neutral red shows a striking change to blue in very acid solutions, but the acidity required is higher than is usually the case with stomach contents.

In conclusion, thanks are due to Dr. J. W. Northington and Miss Swift for aid in the collection of blood samples.

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**THE DIRECT AND INDIRECT CALORIMETRY OF
CASSIOPEA XAMACHANA**

**THE EFFECT OF STRETCHING ON THE RATE OF
THE NERVE IMPULSE**

BY
J. F. McCLENDON

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REPRINTED FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY

VOL. XXXII, No. 2, NOVEMBER, 1917

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and the Physiological Laboratory of the University of
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(Received for publication, September 7, 1917.)

The object of the present paper is to throw light on the factors influencing the oxidation rate of living cells. Henze (1910, a) observed that sea anemones use less oxygen when less is present in the sea water, but interpreted this as due to the time required for diffusion into the animal. That is to say, he supposed that all of the cells were not supplied with oxygen when there was little in the sea water. If oxygen was entirely absent in some of the cells, the decreased oxidation may have been merely the expression of the lesser number of cells taking part in the metabolism. Burrows showed that tissue cells require a certain oxygen tension for growth, and Loeb and Wasteneys found that the heart beat of *Fundulus* embryos may be slowed by reducing the oxygen. The growth of *Fundulus* may be suspended by lack of oxygen and may be slowed by diminishing the oxygen.

EXPERIMENTAL

The calorimeter consisted of either an 840 cc. or a 900 cc. Dewar flask (thermos bottle) enclosed in an air tight container, which was immersed in water that was maintained at the same temperature as the water in the flask, within 0.003°. The technical difficulties were met as follows: Two Beckmann thermometers were adjusted and compared over the range of temperatures of the experiments, and fitted with reading lenses to estimate down to 0.001°. A large tank of sea water was brought to 30° (which was about the temperature of the air) and its pH and O₂ and CO₂ content determined. Some of this sea water was dipped out and a *Cassiopea* in-

roduced into it. The thermos bottle, stopper, and thermometers were immersed in the large tank until they reached the temperature of the water. The *Cassiopea* was transferred to the thermos bottle and a perforated cork stopper inserted with the exclusion of air bubbles. One Beckmann thermometer was inserted through the perforation in the stopper and the other suspended in the tank, near the middle of the thermos bottle. A small hole, remaining in the stopper for exit of displaced water, was closed with wax. The pulsations of the *Cassiopea* stirred the water inside the thermos bottle; and the water in the tank was mechanically stirred and was kept at the same temperature as that inside the thermos bottle by additions of small portions of warmer or colder water as required. The light was excluded by the silvering and coverings of the thermos bottle, but in some experiments, in which a 900 cc. glass jar with ground glass cover was used in place of the thermos bottle, the light could be excluded by darkening the tank, so as to prevent photosynthesis in the symbiotic plant cells. Time was measured by means of a stop-watch and a clock. The same *Cassiopea* was used in a series of experiments.

The oxygen in the sea water was determined by the Winkler method, which can be corrected for the slight error due to a slight amount of organic matter given out by *Cassiopea*, and it was thought impracticable to use the complicated method of Shutzenberger and Risler (Henze, 1910, b). A 250 cc. glass-stoppered bottle was weighed empty and full of distilled water at a known temperature, in order to standardize its volume. It was fitted with a double-bored rubber stopper and a long and a short glass tube with rubber connections. The bottle was filled with mercury and the long glass tube sucked full of the water to be analyzed and the stopper inserted. By inverting the bottle, the sea water was siphoned into it, when the rubber stopper was removed and the glass stopper inserted. The glass stopper was lifted and 1 cc. of alkaline KI solution and 1 cc. of 40 per cent MnCl_2 solution introduced (correction being made for this in the O_2 calculation) and the stopper inserted. Colloidal membranes formed about the drops of alkali and violent shaking was necessary to break them. After the precipitate had settled, 2 cc. of concentrated HCl were added and the stopper inserted and the bottle shaken. Its contents were transferred to a flask and titrated with 0.01 N sodium thiosulfate solution until the yellow color disappeared, then starch solution was added and the titration continued until the blue color disappeared, then the water was poured into the bottle and back into the flask and titrated until the blue color disappeared. The calculation was as follows:

$$\frac{0.056 \times \text{cc. thiosulfate}}{\text{capacity of bottle} - 2} = \text{cc. of oxygen per liter of sea water, and this quotient} \times 0.9 = \text{cc. of oxygen in calorimeter (on the assumption that the concentration of oxygen in the } \textit{Cassiopea} \text{ was the same as that in the sea water).}$$

In order to reduce the carbonate content, the alkaline KI solution was made fresh every few days from two stock solutions. Carbonate-free NaOH solution was made by dissolving 100 gm. of NaOH in 100 cc. H_2O

in a glass-stoppered bottle and pipetting off after the carbonate had settled. One part of this was mixed with one part of 20 per cent KI solution before being used. NaOH made from metallic sodium contained a trace of nitrite and NaOH purified by alcohol contained but little more. This nitrite causes no error if the titration is quickly made immediately after adding the acid, but the contents of the flask slowly turn blue for hours after the end-point has been reached. If the acidity is greatly increased, however, the nitrite causes an appreciable error in the titration.

The MnCl_2 solution contained a trace of Mn(OH)_2 , but this was removed by decantation. The thiosulfate was dissolved in CO_2 -free water and kept in an automatic burette with soda-lime tubes, and standardized with pure iodine. The starch solution was allowed to settle and only the clear upper portion used. When dextrins appeared in it, a fresh solution was prepared.

The number of gm. of chlorine per kilo of sea water (abbreviated Cl) was determined by titrating with a silver nitrate solution standardized with standard sea water from the International Commission. The alkaline reserve was determined by titrating 100 cc. of sea water with 0.01 N HCl, while boiling in a 500 cc. Erlenmeyer flask of resistance glass, using dibrom-*o*-cresolsulfophthalein as indicator, until every trace of purple color had permanently disappeared. The water must not dry on the sides of the flask. The pH was determined colorimetrically by means of the method and identical tubes previously described (McClendon, 1917, b). Correction was made for Cl. It was found that new supplies of indicators must be tested before being used to see that they have the proper pH range. The total CO_2 was determined from the pH and alkaline reserve by means of the conversion table (McClendon, 1917, b).

The weights and one thermometer were standardized by the United States Bureau of Standards and the other apparatus was standardized with them.

Although pH and CO_2 determinations were made in all experiments, it was found that the oxygen absorbed could be determined much more accurately than the CO_2 given out, and the CO_2 determinations are not listed with the experiments, but are collected together in the form of respiratory quotients. The respiratory quotients were 0.7, 0.74, 0.76, 0.84, 0.85, 0.86, 0.88, 0.9, 0.91, 0.92, 0.97, 0.99, 1, 1.02, 1.03, 1.1, 1.15, 1.2. It was impossible to tell whether this variation is due entirely to errors in the CO_2 determinations or whether the respiratory quotient varied. It is improbable, however, that respiratory quotients of 1.2 existed for even short periods of time, and these at least may be considered due to technical errors. According to Mayer (1914) *Cassiopea* lives on animal food exclusively and does not

absorb carbohydrates from its symbiotic algae, since it starves as rapidly in the light as in the dark. It may, however, get some carbohydrates from its animal food, or from glycoproteins during starvation. In this connection it may be of interest to note that *Cassiopea* secreted a mucin-like substance. Since the error in estimating CO_2 production may be 30 per cent in half hour experiments with small *Cassiopeas*, it is convenient to assume that the respiratory quotient is constant and is about 0.95, which is also the average found by Vernon for the hardier species of jellyfish on which he made most of his determinations.

The oxygen consumption is about doubled when the temperature is raised from 20° to 30° , and from results on other animals is probably an exponential function of the difference in temperature. Harvey (Mayer, 1917) found the velocity of the nerve impulse in *Cassiopea* to be a linear function of the temperature, and to increase about 64 per cent on raising the temperature from 23° to 33° . I found the activity of the ganglia (rhopalia) in inducing pulsations of the umbrella to be about doubled with a rise from 20° to 30° in temperature. The data were as follows:

Ce O_2 consumption.		Temperature coefficient for 10° rise.	Pulsations per sec.	
20°	30°		20°	30°
0.698	1.54	2.21		
0.57	1.05	1.84	0.27	0.55

These experiments show the necessity of accurate temperature control and in all of the other experiments in this paper the temperature was measured to the nearest tenth of a degree and maintained to within 0.2° of 30° .

The sea surface is at about the optimum pH for metabolism (usually 8.1-8.3), but the variation in metabolism with variation of pH within the range studied is very slight, as shown by the following table (diameter of *Cassiopea* = 10 cm.).

pH	O ₂ concentration.	O ₂ used.
7.50	4.40	1.85
7.53	4.50	1.95
8.24	4.30	2.79
8.24	4.50	2.87
8.24	4.55	2.43
8.38	4.40	2.07
8.52	3.90	2.28
8.72	3.16	2.59

These variations in oxidation may be due to experimental errors and variation in O₂ concentration, except the first two which show a slight lowering of oxidation when the pH is reduced to about 7.5.

In comparing the rise in temperature in the calorimeter with that calculated from the O₂ consumption, the assumptions were made that the respiratory quotient was 0.95, and that a mixture of proteins, fats, and carbohydrates was burned, giving 6 gm. calories per cc. CO₂, as in the following table.

O ₂	CO ₂	Gm. calories.	
		Determined.	Calculated.
2.18	2.07	12.6	12.4
2.54	2.41	16.2	14.5

In these experiments it was assumed that there was no loss of heat, although some heat must have passed into the thermometer and glass lining of the thermos bottle. The specific heat of sea water and *Cassiopea* was taken as unity because the calculations were not accurate enough to warrant the application of small corrections. The experiments had to continue for several hours in order to obtain an accurately measurable rise in temperature, and the tedium of keeping the tank at the same temperature as the calorimeter necessitated the substitution of indirect calorimetry in the remainder of the experiments.

The preliminary experiments to show the effect of O₂ concentration are as follows:

Average O ₂ concentration.....	11.9	1.91	3.36	1.55
O ₂ consumption.....	2.6	2.38	2.26	1.65

In performing these experiments, a number of possible sources of error were thought of and it was decided to make a more detailed study of the metabolism of *Cassiopea* before returning to the subject. The chief danger of error was in prolonging the experiment until all of the O₂ was used up. It was found that *Cassiopea* could live more than 7 hours without oxygen, in which case no measurable quantity of CO₂ was produced. Vernon observed practically no increase in the respiratory quotient of jellyfish correlated with oxygen-want, whereas the respiratory quotient of fishes increased under these conditions.

In order to determine whether the rate of oxidation depends on the oxygen concentration it is desirable to know something about the oxygen concentration inside the living cells. In other words, the transfer of oxygen to the cells must be facilitated as much as possible if we are to judge anything about the concentration of O₂ within them from that in the sea water. This could be approximated by agitating free cells or a single layer of cells with the water or circulating the water over a single layer of cells. When using free cells, some are liable to injury and more or less disintegration, thus interfering with the titrations, but notwithstanding the criticism of Heilbrunn, comparative results may be obtained (McClendon and Mitchell). *Cassiopea* was chosen, because the cells are spread in thin layers on the surface of a mesoglea which will be shown to use practically no oxygen. The pulsations of the *Cassiopea* (Mayer, 1906) bring currents of water over the cell layers, so that diffusion is necessary only for a minute distance. The error due to this diffusion would be large only when the O₂ concentration is very small. By skilful manipulation, the mucous secretion may be prevented from increasing or leaving the surface of the *Cassiopea*.

Evidence that oxidation is confined to the cell layers is apparent in the fact that oxidation is not proportional to the volume but to the surface. It would be practically impossible to measure the surface, but since the individuals are practically of the same shape, the surface is proportional to the square of

the diameter. Since *Cassiopea* is elastic, the diameter was always measured under the same conditions; i.e., resting on a glass plate, with the exumbrella in contact with the glass (and the average of two diameters at right angles to one another taken). Some rough preliminary determinations showed the O_2 consumption in cc. per hour to be about $0.023 \times$ the square of the diameter in cc. as shown in the following table.

Diameter in cm.....	3.5	7	8.5	9.5	10	11.5
O_2 per hr.....	0.4	1.4	1.17	2.16	2.7	2.78

Very small *Cassiopeas* used more O_2 than calculated from the formula (an anomaly which is correlated with more rapid pulsations). A *Cassiopea* 3.5 cm. in diameter pulsated once a second whereas one 10 cm. in diameter pulsated 0.3 times per second. In order to compare experiments on *Cassiopea* where the weight is recorded, it is convenient to know that the diameter in cm. $= 2.25 \times \sqrt[3]{\text{of the weight in gm.}}$.

The following table gives the respiration rate under different conditions, except that the temperature is always 30° .

Diameter.	O_2 per hr. square of diameter	O_2 per liter.	pH	O_2 per hr.
cm.				
7	0.0200	4.5	8.20	1.3
8	0.0187	4.2	8.22	1.26
8.5	0.0152	3.32	8.22	1.09
10	0.0287	4.5	8.24	2.87
10	0.0279	4.3	8.24	2.79
10	0.0243	4.55	8.24	2.43
10	0.0185	4.4	7.50	1.85
10	0.0195	4.5	7.53	1.95
10	0.0207	4.4	8.38	2.07
11	0.0228	3.9	8.52	2.28
10	0.0259	3.16	8.72	2.59
11	0.0230	3.54	8.17	2.79
11	0.0200	1.8	8.40	2.43
11	0.0160	1.57	8.02	1.93
11	0.0320	7.2	8.47	3.84
11	0.0400	7.44	8.46	4.87

In the above table the pH and O_2 per liter at the beginning of the experiment are given and the pH was about 0.09 and O_2 1.5 cc. lower at the end of each experiment. The average O_2 during each experiment influences the O_2 used per hour, but apparently no difference in the quotient of the O_2 used per hour divided by the square of the diameter (second column) can be correlated with difference in size. Using greater extremes of size, however, the quotient seems to decrease as the diameter increases, and therefore extreme sizes were usually avoided.

All of the experiments were made under conditions of starvation and hence the *Cassiopea* used its own substance as a source of energy. Starvation can hardly be considered a pathological process in *Cassiopea*, however, since it may remain alive for months without food, constantly decreasing in weight until it almost disappears before death. Mayer (1914) determined the loss in weight as about 5.6 per cent per day at about 30° , although no thermostat was used. If y is the weight at any moment and w is the weight when starvation commenced and n is the number of days of starvation,

$$y = w (1 - 0.056)^n$$

Since I found the diameter to be 2.25 times the cube root of the weight, if the weight were 100 gm., the diameter would be 10.45 cm. The O_2 consumed during 1 day would be about 0.023 times the square of the diameter times 24 = about 60 cc. O_2 absorbed and 57 cc. CO_2 given out. If we assume that protein was burned and that 5.9 gm. calories correspond to 1 cc. CO_2 , the metabolism would equal 336 gm. calories for the day. If we assume a certain mixture of proteins, fats, and carbohydrates were burned and 6 calories correspond to 1 cc. CO_2 , the metabolism would equal 342 calories per day.

Since I have shown that the metabolism is proportional to the surface and Mayer has shown that the loss in weight is proportional to the volume (weight), the composition of the *Cassiopea* must change during starvation. In other words, it loses weight faster than it burns protein (or other organic matter), and hence the concentration of the protein must increase. Mayer (1914) found the cellular layer did not decrease in thickness during starvation, and Hatai found the percentage of ni-

trogen to the total body weight increases during starvation, and is also greater in small than in large, well nourished *Cassiopeas*. Therefore in attempting to calculate the metabolism from the loss in body substance, we should make it proportional to the loss in surface rather than loss in volume, since the loss in living matter seems to be proportional to the loss in surface, and the *Cassiopea* seems to have no other important store of food than its own protoplasm, the mesoglea apparently functioning chiefly as a skeleton. Since the surface is proportional to the two-thirds power of the volume, we may assume that the protein is proportional to the two-thirds power of the weight (the density remaining practically constant). The protein equals 5.16 per cent of the two-thirds power of the weight (calculated from Hatai's data on the assumption that protein is 16 per cent N). The weight at the beginning of starvation was 100 gm. and the protein 1.107 gm.; at the end of 1 day the weight was 94.4 and the protein 1.07, being a loss of 37 mg. of protein. If we assume that 1 mg. of protein is equivalent to 4.4 calories the metabolism the 1st day would be 163 calories, although I found it to be 336-342 calories. Although these calculations are only approximate, since starvation is a little greater the 1st day than calculated by the formula, this great difference indicates that the burning of protein does not account for all of the heat. Since living cells contain lipoids or lipo-proteins, and carbohydrates or glyco-proteins, it seems probable that proteins, carbohydrates, and lipoids are burned. The mesoglea has not been analyzed separately, but is largely sea water, with possibly a trace of glyco-protein. It probably has little calorific value, since the use of a store of food would cause a relatively greater metabolism in large starving *Cassiopeas* than was actually observed.

Since the lining of the alimentary tract is not at the surface of the *Cassiopea* and O_2 must diffuse through at least a mm. of tissue to get to it, it was decided to pull off the manubrium and study the metabolism of the umbrella. The umbrella is disc-shaped and covered on both sides by epithelium, and pulsates, thus circulating the water. The wound made by removal of the manubrium is of small area and is covered by an epithelium within a few hours, and the umbrella will live as long as a starving *Cassiopea*. Some rough determinations indicate

that the respiration of the umbrella is only about a fourth as great as that of the whole *Cassiopea*. In the following table are recorded measurements on three *Cassiopeas* and on their umbrellas after removal.

Diameter.	O ₂ per hr. square of diameter		Pulsations per sec.
	<i>Cassiopea</i> .	Umbrella.	
cm.			
3.5	0.033	0.0075	0.94
9.5	0.021	0.0041	0.58
11.5	0.030	0.0075	0.63

Since the respiration is influenced by the muscular activity or pulsation rate and the latter is not constant, it was decided to remove the ganglia (rhopalia) that induce the pulsations and start a continuous contraction wave running around the subumbrella, the middle third of which has no neuromuscular tissue (Mayer, 1908). The rhopalia were cut out by means of a cork borer and the wave was started by electrical stimulation. It was noticed, however, that the contraction wave, apparently constant for short intervals of time changed more rapidly at first and then more slowly, but never became absolutely constant, the change being perhaps associated with shrinkage of the umbrella. The effect of shortening and stretching on the contraction wave was therefore studied. The rate of the contraction wave depends on the rate of the nerve impulse around the circuit of the nerve-muscle layer, but does not depend solely on the rate in the neuraxon, since there are numerous synapses, and furthermore, the path of the impulse is zigzag. Professor Cary kindly showed me a stained preparation of the nervous network of the subumbrella. Concentric rings cut from the umbrella are capable of maintaining a trapped wave for some time, but if the ring is too narrow, the wave cannot be started, or soon ceases after being started. Trapped waves can be started in two or three concentric rings cut from the umbrella and the wave revolves about the inner ring more often per second than about the outer ring, but the revolutions per second are not in exact inverse proportion to the mean diameters of the rings, or to the diameters of the inside tracks or holes in the rings. One subumbrella, 11.5 cm. in diam-

eter, was cut into two rings and waves were trapped in them. The wave in the inner ring made 2.5 revolutions per second, and the wave in the outer ring made 2 revolutions per second.

The uncertainty as to the length of the pace-making circuit that the nerve impulse takes around the ring may be avoided by stretching the inner edge of the ring until it is of the same diameter as the outer, thus transforming the ring into a cylinder or belt. Such a ring can be stretched further and behaves in a strikingly reversible manner. Since Mayer (1917) has shown that the rate of nerve conduction in *Cassiopea* depends on temperature and electric conductivity of the sea water, it should be noted that all the experiments in this paper, unless otherwise stated, were done in sea water of 30° and Cl = 20. Mayer found a variation of only about 2.5 per cent over the range of pH = 5.6-8.26, and this variation includes experimental errors and changes due to unknown causes. In the present experiments the pH was about 8.2 unless otherwise stated. The only difficulty in estimating the rate of the contraction wave arose from the fact that the rate is 1 to 5 per cent faster in the ring that has just been stretched than in the ring that has just been relaxed, depending on the degree of recent stretching or relaxation. If, however, the circumference of the ring is allowed to remain constant for 5 minutes after each short step of stretching or relaxation, the rate will approximate a mean value. This behavior of the ring may be regarded as a form of hysteresis, since the number of revolutions per second of the contraction wave tend to remain constant immediately after stretching or relaxation. I do not see how this can be explained on the assumption that the stretching of the neuraxon is the only factor, and it is significant to note that Carlson records no such hysteresis in the stretched nerve of the slug, where synapses are less numerous or entirely absent. One explanation of the increased rate immediately after stretching might be the thinning of the plasma membrane of the neuraxon, the regeneration in thickness taking appreciable time. Another suggestion is made that the immediate effect of stretching is increase in length of the neuraxon, but that this may be proportionately less than the increase in length of the strip of tissue, since the nerve paths may be straightened, and that the apparent hysteresis is due to the possibility that after the nerve is stretched synapses gradually

open, due to the tension, until the nerve paths are proportioned as zigzagged as at the start. This supposition may seem to be applied to the fact that by prodding a *Cassiopea* (having a trapped wave) with a stick, the number of revolutions per second is reduced, although no apparent increase in length of the conducting path remains. The local stretching of the subumbrella with the stick might break some of the nerve apses. Such complicated suppositions are not very probable, however, since they are not easily tested. A more probable hypothesis is given below in connection with the amplitude of the contraction wave. The significant fact is that (ignoring the period of readjustment) the ring may be stretched until its circumference is increased 72 per cent with practically no change in rate (mm. per second), although in order to accomplish the number of revolutions per second or passages of the wave through the same tissue may be reduced 49 per cent. This is analogous to the effect of stretching a metallic wire on the passage of an electric current through it, with the difference that the process is completely reversible in *Cassiopea* after an increase of 72 per cent in length due to stretching. The results of the rings are as follows:

Length of circumference.	Rate (mm. per sec.)
mm.	
223	372
263	413
283	430
286	379
306	390
326	399
346	410
366	414
386	407
406	403
426	391
446	377
466	368
486	360
506	352
526	342

If the rate of wave propagation is the same, we would expect in umbrellas of *Cassiopeas* of different sizes that the number of revolutions of the wave per second would be inversely proportional to the diameter. The diameter is measured before the wave is started, and there is a progressive shrinkage in the diameter, due to starvation and decrease in volume, tension of the regenerating tissue after removal of the rhopalia, and increased tonus of the musculature (transforming the disc into a cup shape). This decrease in the diameter is associated with increase in revolutions per second, but agitation decreases the revolutions per second. It is therefore necessary to make the determinations under the same conditions for strictly comparative results. The following determinations were rough, but serve to indicate the general features.

Diameters.	Revolutions of wave per sec.		
	Immediately.	After 24 hrs.	After prodding with a stick.
cm.			
9.75	2.38	2.50	
10.50	1.79	2.17	2.04
10.50	1.75	2.17	2.04
10.75	1.75	2.13	
11.00	2.38	2.08	
11.50	1.72	2.22	
11.50	1.79	2.22	
12.50	1.67	1.72	1.66
12.80	1.67	2.00	
13.50	1.61	1.67	1.40
13.50	1.61	1.79	1.52
14.00	1.67	1.79	

A comparison of these experiments with those on the actual velocity of the wave shows the circumference of the potential pace-making circuit to be about 1.8 times the diameter. If the contraction wave is stopped by pressure and after a rest of some minutes or hours started again, it is slower than just before stopping, but if it is started again as quickly as possible after stoppage, the rate is the same. This effect of a rest seems to be associated with nutrition or recovery from fatigue, since the amplitude of the contraction wave is greater in the rested

umbrella, although the number of revolutions per second is decreased. Whether the actual rate of propagation is changed would be difficult to determine. It seems evident that the wave of nerve impulse precedes the wave of muscular contraction. The contraction of the muscle must stretch the adjacent regions, and hence stretch the region through which the nerve impulse is passing, thus increasing the distance traveled in one revolution and decreasing the revolutions per second. When the amplitude of contraction is increased, the stretching of the nerves is increased and the revolutions per second are decreased, but whether this can account for the total decrease has not been determined. The speeding up of the revolutions per second after the trapped wave is started is at first more abrupt and later more gradual, and is associated with both decrease in amplitude of contraction and decrease in diameter of the umbrella, due to starvation and contraction of scar tissue.

Evidently a change in the number of revolutions per second of the trapped wave or the amplitude of the contractions would cause an error in the determination of the effect of O_2 concentration on metabolism, and in order to estimate the limits of such errors, the relative metabolism of the muscle and other tissues was studied. The umbrella of three *Cassiopeas*, *A*, *B*, and *C*, of the same size (diameter = 11.5 cm.) were used (each for a series of experiments). In some experiments the rhopalia remained and the normal pulsations were generated, in others a trapped wave was induced, and in others the subumbrella was removed or merely the mesoglea left. The apparent (but slight) metabolism of the mesoglea was probably entirely due to a few remnants of epithelium and to bacteria, which always attack the mesoglea when the epithelium is removed. At any rate the metabolism of the mesoglea is too small to be of significance. The pH was 8.2 and the O_2 per liter 4.5 cc. at the beginning of each of the experiments.

The removal of the rhopalia in the umbrellas with trapped waves or without pulsations, reduced but slightly the amount of tissue. If we take the metabolism of the normal pulsating umbrella as 100, the exumbrella is about 14 and the resting subumbrella 60 with an addition of 26 for normal pulsations or 65 for trapped wave. Therefore, the neuromuscular tissue may per-

Individual.	O ₂ per hr.	Parts of umbrella used.
	cc.	
A	0.86	Umbrella with 0.64 pulsations per sec.
A	0.97	" " 0.64 " "
A	0.158	Mesoglea + exumbrella only.
B	1.12	Umbrella with trapped wave, 1.47 per sec.
B	1.08	" " " " 1.47 " "
C	0.5	" not pulsating.
C	0.6	" " "
C	0.03	Mesoglea + bacteria.
D	0.88	Umbrella with 0.7 pulsations per second.
D	0.7	" not pulsating.

form about 26 to 46 per cent of the metabolism, and changes in rate or amplitude of the contraction wave are to be avoided as much as possible. In subsequent experiments, the manubrium and rhopalia were removed from the *Cassiopea*, and a C-shaped cut was made through the neuromuscular layer about one-third the radius from the outer margin of the umbrella, and a trapped wave induced by stimulation near the outer margin. The wave passed around the outer part of the subumbrella, and each time it passed the opening of the C it spread to the inner part, dividing into two equal waves, meeting on the far side with mutual destruction. In this way, the outer part, in which the trapped wave was first induced, was made pace-maker, and the result was a more permanent wave. The wave causes circulation of water against all parts of the epithelium except a small portion of the exumbrella which has a very low metabolism. If the oxygen concentration was reduced to zero, the wave stopped, and metabolism ceased until oxygen was readmitted. In one experiment an umbrella was kept 7 hours at zero O₂ concentration, then 3 hours with O₂ and a trapped wave, and 7 hours at zero O₂ concentration, during which it gave out no CO₂ or other acid products affecting the pH perceptibly. 30 seconds after it was taken out of the O₂-free chamber, a trapped wave was started and this constantly increased in amplitude for 10 minutes, at the end of which time the amplitude was normal. Oxygen was removed from sea water in various ways, with the air pump and agitation, by boiling, and by allowing a *Cassiopea* to remain

in it until the pulsations ceased, as seen through a peep hole in such a way that photosynthesis was practically avoided. In no case did the analysis show less than about 0.05 cc. per liter, but that amount probably entered with the KI and MnCl_2 solutions and around the ground stopper of the analysis bottle. At any rate, we should consider 0.05 cc. to be within the limit of error of the method if no correction be made for O_2 in the reagents.

The variation in metabolism after removal of the manubrium and initiation of a trapped wave is shown in the following table. The diameter of the umbrella was 11.5 cm. at the beginning of the first experiment and at the beginning of each experiment the pH was 8.2 and the O_2 per liter 4.5 cc.

Hrs. after operation.	O_2 used per hr. cc.	Revolutions of wave per sec.
0	1.65	1.60
2	1.55	2.00
3	1.30	2.22
4	1.30	2.22
5	1.30	2.22
20	1.30	2.22

From the above table it is evident that the metabolic rate may vary rapidly for 3 hours after the manubrium is removed and the trapped wave is started, therefore in the subsequent experiments, the umbrella was not placed in the respiration chamber until these 3 hours had passed. The same umbrella used in the above experiments (but 21 hours after the operation) was used to determine the effect of reduced oxygen concentration. The average O_2 concentration during the experiment was 1.5 cc. per liter and the O_2 used per hour 1.03 cc., being a decline of 20 per cent in rate of oxidation with a reduction of the oxygen concentration to about half its original value. This and some later experiments are tabulated as follows:

3.3 cc. O ₂ per liter. O ₂ used per hr.	At 1.5 cc. O ₂ per liter. O ₂ used per hr.
1.3	1.03
1.3	1.00
1.4	1.10
1.48	1.15
1.44	1.25
1.35	1.05

terminations show the oxidation is reduced about 20 per cent when the O₂ concentration is reduced about 50 per cent, and oxidation increased about 25 per cent when the O₂ concentration is increased about 100 per cent.

DISCUSSION.

Our experiments show that the rate of oxidation varies with the concentration of oxygen in the sea water constantly against the surface of the epithelium in which oxidation takes place. In the total absence of oxygen, no measurable amount of CO₂ or other acid products is given out, and we find that the metabolism is suspended. If oxygen is readmitted, a suspension of the metabolism for 7 hours, the rate of metabolism rises apparently to the normal within 10 minutes. No anaerobic processes were detected during the absence of oxygen for 7 hours, but in the absence of oxygen for 16 hours, anaerobic or hydrolytic processes take place. No evidence was found to indicate that these anaerobic processes constituted the main part of the metabolism of the *Cassiopea*. On the contrary, a great number of bacteria was associated with them, and the *Cassiopea* rapidly dissolved and could not be revived by readmission of oxygen. We may assume that oxygen protects the *Cassiopea* against attacks of bacteria (probably anaerobes). Since the distance from the surface, it seems probable that the *Cassiopea* might live indefinitely on so small a supply of oxygen as is used in the superficial cells as fast as it diffuses into the tissue, none reaches the deeper cells. If this be true, the rate of oxygen consumption with variation of supply might be a function of a variation in the number of cells receiving oxygen. In other words, this would be a diffusion phenomenon.

Another hypothesis is that the concentration of oxygen at the seat of oxidation affects the rate. Warburg has shown that cell oxidation is associated with structure and that no enzyme solution has been obtained that will account for the vital oxidation of foodstuffs. If we call the structure or surface responsible for the oxidations the catalyst, it seems possible that the concentration of oxygen in the immediate vicinity of the catalyst influences the rate. This influence of concentration on rate might still be a diffusion phenomenon since the O_2 must diffuse toward the structure catalyst or oxidase molecule.

Owing to the excellent review of the literature on the subject of this paper by Krogh, it seems unnecessary to multiply references. Roughly speaking, and within physiological limits, animal oxidation is about doubled with 10° rise in temperature and this is shown here to be true of *Cassiopea*. In other words, oxidation is an exponential function of the temperature, as expressed in the following equation:

$$V_t = V_0 \times 2^{\left(\frac{t}{10}\right)}$$

Where V_t is the velocity of oxidation at t° and V_0 at 0° . This relation holds approximately true for a number of chemical reactions, and is supposed to be due partly to change in diffusion rate and partly to loosening of bonds in the reacting molecules, and to ionization. Diffusion depends on viscosity and molecular motion. A fall of temperature from 30° to 20° increases the viscosity of water about 25 per cent, and of a 40 per cent sucrose solution 42 per cent and of a 3 per cent gelatin solution 1,000 per cent (von Schroeder). The increase in viscosity of gelatin, however, does not cause a proportionate decrease in diffusion. We may assume that this fall of temperature may possibly cause about 30 per cent decrease in diffusion of O_2 within the cell, due to viscosity alone. Molecular motion is proportional to the absolute temperature, and hence is a minor factor in diffusion. Although diffusion accounts for only about a third of the change in rate of oxidation with temperature, it is undoubtedly a factor. Since diffusion affects oxidation and concentration gradient affects diffusion, it seems very probable that concentration of O_2 should affect oxidation, even though every cell received some

oxygen. Krogh interprets the experiments on warm-blooded animals as showing increased oxidation with increased oxygen tension, and reviews the work of Thunberg, showing the same effect to a greater degree on cold-blooded animals. Henze (1910, a) found that the oxidation rate of sea anemones and annelids varies with O_2 concentration (and of nudibranchs at low concentrations) and supposed that only a varying fraction of the cells received oxygen. His tables show that the oxidation rate of crustacea and jellyfish fell rapidly with time, but that in one series of determinations on *Pelagia*, the oxidation rate varied reversibly with O_2 concentration. In order to reduce the diffusion effect, he kept sea urchin eggs agitated in sea water and found that the oxidation rate apparently increased about 8 per cent on doubling the O_2 concentration. Henze attributes this apparent difference in respiration to faulty technique, but since the experiments have not been repeated with improved technique, we may assume that a real difference exists.

It is interesting to compare the metabolism of *Cassiopea* with that of jellyfish studied by Vernon. Since only the living cells metabolize, it would be an advantage to know the proportion of cellular tissue to the body weight, but there is no data on this subject. The skeletal structure, mesoglea, contains less organic matter than the cells, and hence the per cent of organic matter in the body is a partial indication of the cellular mass. The proportion of mesoglea increases with the size of the individual within the same species, but there can be no strict comparison between different species in this regard, and therefore the comparison is very crude. The number of cc. of O_2 per hour per kilo of body weight and per kilo of organic matter (= dry weight — weight of salts in equal volume of sea water) at 20° is given in the following table.

Species.	Weight of individual.	Cc. O_2 per hr.	
		Per kilo of body weight.	Per kilo of organic matter.
	gm.		
<i>Cassiopea xamachana</i>	100	12.5	1,040
<i>Carmarina hastata</i>	30	7.7	2,025
<i>Cestus veneris</i>	70	3.75	1,562

The agreement is about as close as could be expected.

Vernon has compared the metabolism (per unit weight of organic matter) of jellyfish, molluscs, tunicates, and vertebrates, and shown it to be remarkably constant. Krogh obtained greater differences when the total body weights were used (omitting jellyfish but including eggs and insects), but the differences probably do not exceed the differences in water content and in muscular activity. He found the metabolism of a young dog with body temperature reduced to 20° to be greater than that of cold-blooded animals at the same temperature, but if we reduce the metabolism of the average dog for 20°, using a reasonably high temperature coefficient, the agreement is more satisfactory. We should not expect close agreement unless water and mineral salts and fibrous tissue are excluded from the weight, and the activity of the nervous system is abolished. It seems probable, therefore, that the chief distinction in the calorimetry of warm-blooded and cold-blooded animals is in insulation. All warm-blooded animals are air breathing, and air brings much oxygen and takes away little heat. The center of a cluster of bees in winter may be 40° above that of the air bathing it.

The heat production in a 100 gm. *Cassiopea* at 30° is sufficient to raise its body temperature 0.14° per hour above that of the surrounding water, but no such difference in temperature has been observed because the heat generated is conducted away by the water bringing the oxygen. I found a fish weighing 1.4 gm. to use 0.825 cc. O₂ per hour at 30°, which is sufficient to raise its body temperature about 3° per hour, but during this time it was required to breathe 400 cc. of sea water, even though it removed half of the oxygen from water saturated with air at this temperature. The water circulating through the gills could remove the heat generated if the body temperature were 0.01° above that of the water. Since there was considerable conduction through the skin, and the fish probably removed much less than half the oxygen from the water in one passage through the gills, the body temperature was probably much less than 0.01° above that of the water. Rogers and Lewis could detect no difference between the body temperature of fish, salamanders, clams, and earthworms and the water in the thermostat in which they were placed, after they had been in the thermostat

long enough for equilibrium. They used a thermocouple, and one division of the galvanometer scale corresponded to 0.0042° .

It was shown that the metabolism of *Cassiopea* is proportional to its surface and not to its weight but to $W^{\frac{2}{3}}$. This is due to the fact that the metabolism is confined to the living cells and that these constitute a superficial epithelium, whose thickness is about the same in *Cassiopeas* of the range of sizes studied. We might use these results in an attempt to explain the so called surface law of warm-blooded animals. Dreyer, Ray, and Walker have shown that the blood volume and cross-sections of the aorta and trachea are proportional to $W^{\frac{2}{3}}$ (or surface). If animals are of the same shape (internally as well as externally) the cross-sections of all organs would be proportional to $W^{\frac{2}{3}}$ but the blood volume would be proportional to the weight (W). If the blood volume is proportional to $W^{\frac{2}{3}}$ the whole circulatory system would be nearly proportional to $W^{\frac{2}{3}}$ and owing to the close relation between the lungs and the blood, the volume of the lungs would be nearly proportional to $W^{\frac{2}{3}}$. The volume of the skin may be proportional to $W^{\frac{2}{3}}$ and the volume of the wall of the alimentary tract nearly so. The nerve, muscle, and glandular tissues are excitable, and hence their metabolism must vary. Variable components may be excluded from basal metabolism by definition but cannot all be excluded in making measurements. Only the skeleton can be said to have a metabolism proportional to W , and since the red bone marrow produces blood (erythrocytes) and this is proportional to the surface, the metabolism of some of the bones may be nearly proportional to the surface. Benedict has shown that great variations from the surface law exist, and hence it may be only accidental. The fact that the excitable tissues metabolize more per unit weight in small animals than in large (i.e., proportional to a smaller power of W than unity) is true, not only for warm-blooded animals, but also for cold-blooded animals, to which the teleological principle of the surface law (in relation to heat regulation) does not apply. It seems possible, however, that the chief conditions necessary for the evolution of temperature-regulation in animals were: (1) air breathing, (2) large body size, (3) sensitivity to low temperatures, (4) variation of activity of excitable tissues with size, and

(5) epithelial type of architecture, (4) and (5) being common to cold-blooded animals.

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THE WAVERLY PRESS
BALTIMORE, U. S. A.

1917

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DIURNAL CHANGES IN THE SEA AT TORTUGAS, FLORIDA

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Communicated by A. G. Mayer, September 21, 1917

The only diurnal change noted in the Gulf Stream was a change in temperature of about 1° and the resulting change in oxygen tension. But in water shallow enough for considerable light to reach the bottom, marked diurnal changes were noted in temperature, hydrogen ion concentration (pH), total CO_2 -concentration, CO_2 -tension, O_2 -concentration and O_2 -tension. The temperature, O_2 -concentration and O_2 -tension were lowest and the CO_2 -concentration and CO_2 -tension highest about 5 a.m. The temperature, O_2 -concentration and O_2 -tension were highest and CO_2 -concentration and CO_2 -tension lowest at about 3 p.m., local apparent time during July. The magnitude and exact time of maxima and minima varied somewhat from day to day and varied a great deal with the location of the station at which the water was studied. The diurnal curves showed secondary notches which were probably due to tidal currents and eddies, since no such notches were present in the diurnal curves of stagnant sea water. The differences between stations were evidently due to previous history of the water carried past the station by currents and to variations in depth and in

fauna and flora at the bottom. The details of this work, including maps, graphs and tables, are to be published by the Carnegie Institution of Washington. The relation of local conditions to the precipitation of CaCO_3 , thus decreasing the depth of the water, is pointed out.

Studies of the effect of these changes on organisms were made. The limiting factor for plants seems to be fixed nitrogen. Only 0.02 mgm. of fixed nitrogen per liter could be determined and it was not thought practicable to determine local changes with certainty. The limiting factor for animals seems to be food. Oxygen could easily become a limiting factor. One kilogram of fish would use up all of the oxygen in 4300 liters of water of the lowest O_2 -concentration found at the surface, in twenty-four hours. It seems improbable that fish alone would suffocate, but swarms of Dinoflagellates might suffocate themselves and other animals present.

THE EFFECT OF OXYGEN TENSION ON THE METABOLISM OF CASSIOPEA

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Communicated by A. G. Mayer, September 21, 1917

It was shown by Verzár (*J. Physiol.*, 45, 1912, 39) that decreased oxygen tension in the blood capillaries decreased the metabolism of muscle but not of salivary glands. The animal died before the oxygen tension in the salivary glands was reduced sufficiently to cause a noticeable fall in metabolism. In order to avoid complications in circulation, as in Verzár's experiments, I used the umbrella of *Cassiopea* in such a manner as to maintain a thin layer of cells of uniform activity constantly bathed with sea water at 30°. It had been determined in preliminary experiments that a rise of 10° in temperature doubled the metabolism, but that the hydrogen ion concentration could be changed within certain limits without changing the metabolism to a degree that could be measured with certainty. The average of a large number of determinations placed the respiratory quotient at 0.95, but whether it was constant could not be determined. From the heat produced and nitrogen lost it was concluded that proteins with a small admixture of carbohydrates and fats were burned.

Since the temperature was constant, the oxygen-tension was proportional to the O₂-concentration, as determined by the Winkler method, the mean of the values at the beginning and end of the experiment being used. The metabolism was measured by the oxygen used, as that was determined more accurately than the heat and CO₂ eliminated. *The metabolism varied with oxygen concentration.* This may be true of the cells of all animals. It seems possible that Verzár did not succeed in markedly changing the O₂-tension in the salivary gland, owing to the great store of oxygen in the hemoglobin. There is, however, a distinction between the metabolism of vertebrate muscle cells and *Cassiopea*. If vertebrates are asphyxiated, the muscles give out lactic acid. A *Cassiopea* may remain without oxygen for seven hours without giving out CO₂ or any other acid causing a noticeable change in hydrogen ion concentration, although in the presence of O₂ such a change appears in

a few minutes due to elimination of CO_2 . After seven hours without O_2 , nerve conduction and noticeable contractility of muscle returns in thirty seconds after suspending the *Cassiopea* in air.

Since O_2 -tension may affect metabolism, it seems probable that changes in the threshold of stimulation of the respiratory and vasomotor centers may affect metabolism in man and mammals. The details of the experiments will be published elsewhere.

THE EFFECT OF STRETCHING ON THE RATE OF CONDUCTION IN
THE NEURO-MUSCULAR NETWORK IN CASSIOPEA

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Communicated by A. G. Mayer, September 21, 1917

It was observed by Carlson (*Amer. J. Physiol.*, 27, 1911, 323) that stretching the nerve of the slug has no effect on the rate of the nerve impulse. This does not support Bethe's hypothesis that the impulse passes over solid neuro-fibrillae which are zig-zagged in the relaxed nerve and straightened out in the stretched nerve. Conditions are not so simple in the nervous network of the sub-umbrella of Cassiopea, but the rate may be more accurately determined. The stretching may increase the original length 84%.

A ring or belt of umbrella tissue was placed in a frame by means of which the circumference could be stretched and at the same time its length measured. The apparatus was immersed in sea-water kept at 30°. A neuro-muscular wave was started in the ring in such a manner that it traveled continuously around the ring and its speed measured by noting the time at which the muscular contraction wave passed a certain point. The time required for the wave to pass 100 times around the ring was recorded with a stop-watch. An example of one of the experiments is as follows:

Length of circumference, mm.	286	306	326	346	366	386	406	426	446	466	486	506	526
Rate (mm. per second).....	376	390	399	410	414	407	403	391	377	368	360	352	342

It may be noted that the rate changed 17% while the length increased 84%, in other words the rate is relatively constant. An uncertainty of rate of 1-5%, due to hysteresis, could not be analyzed with certainty owing to the fact that the course of the neuraxones is zig-zagged and interrupted by synapses, but some speculation on this phenomenon will be published elsewhere. The purpose of this abstract is to point out that the experiments on Cassiopea tend to support Carlson's conclusion that stretching the nerve does not change the rate, and that the conducting substance, itself, can be stretched and relaxed.

London

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1

THE EQUILIBRIUM OF TORTUGAS SEA WATER WITH CALCITE AND ARAGONITE

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Communicated by A. G. Mayer. August 30, 1917

The question of the solubility of calcite and aragonite in sea water is a matter of interest in relation to the geology of limestone and dolomite. Murray and Hjort¹ maintain that sea water is so complicated a mixture that the solubility of CaCO_3 cannot be calculated with certainty (from the law of mass action) but that the experiments of Anderson and of Cohen and Rahen show that sea water is saturated with calcite. They add, (p. 181) that dolomite is less soluble than calcite in carbonated waters. Their book summarizes observations showing that calcium carbonate is precipitated in shallow tropical waters, but that even shells are dissolved in the red clay bottoms of the depths.

Mayer² placed pieces of *Cassis* shell in sea water for more than a year and found them to maintain their weight within about $\frac{1}{10}$ of 1%. The precipitation of CaCO_3 at Tortugas was studied by T. Wayland Vaughan, R. B. Dole, and G. H. Drew.³ Drew observed that a denitrifying bacillus, *Pseudomonas calcis*, obtained from the sea water, was capable of changing calcium nitrate to calcium carbonate in culture media and supposes a similar process to occur in sea water. Since Vaughan has observed that calcium carbonate is constantly precipitating at Tortugas, Drew's hypothesis necessitates the presence of an appreciable amount of nitrates or nitrites, and I have attempted to determine them.

A half liter of sea water was boiled in an all-glass still and the distillate collected in a series of 25 cc. Nessler's tubes. Another series of Nessler's tubes were filled with a graded series of concentrations of ammonium chloride. One cubic centimeter of Nessler's reagent was added to each tube and agitated. After fifteen minutes, the tubes were compared colorimetrically and the ammonia recovered from the sea water was estimated. After no more ammonia could be distilled from the sea water, amalgamated aluminium shavings were introduced into the still and the distillation process repeated. The ammonia recovered was formed by reduction of nitrates and nitrites. Duplicate analyses gave less than 0.01 mgm. of nitrogen per liter as ammonia and less than 0.01 mgm. nitrogen per liter as nitrates and nitrites. Raben found more than ten times these quantities in North Sea water.⁴ Evidently, *Pseudomonas calcis* and other organisms have almost completely removed the fixed nitrogen from Tortugas sea water. The effect of this probably explains the scarcity of life in the vicinity of Tortugas as compared with colder seas (law of minimum). There is, however, a constant renewal of fixed nitrogen from the atmosphere, from the decay of organisms and probably from water rising from the depths of the ocean. If *Pseudomonas calcis* is an important agent in the precipitation of CaCO_3 , its action is evidently more intense in places where calcium salts, nitrates and nitrites are carried from the land into the sea.

That calcium carbonate is withdrawn from surface waters of the sea, is shown by chemical analyses. Dittmar⁵ found an average of 0.44% less calcium in surface waters than in deeper waters. This is true notwithstanding the fact that calcium carbonate is constantly being added to the surface waters. The drainage of the land contains an excess of calcium carbonate and flows out on the surface of the sea, where the water evaporates leaving the excess of CaCO_3 in the sea water. The action of organisms in building calcareous structures may account for a large part of the depletion of surface waters, but the precipitation of calcareous mud at Tortugas has been observed by Vaughan.

The analysis of the calcium content of sea water requires double precipitation, and filtration for separation from magnesium and hence large samples and great care are required for accuracy. Theoretically, however, we may detect differences in calcium content by titration. Dittmar showed that except for H_2O , calcium and gases, sea water is remarkably constant in composition. The water content may be determined by titration of the chlorides and the gases may be eliminated by boiling after the addition of enough acid to decompose the carbonates. If we disregard carbonic acid, there is an excess of bases

in sea water, i.e., the sum of the base equivalents is greater than the sum of acid equivalents. Since calcium is added to or taken from sea water in the form of CaCO_3 , and change in the calcium content causes an equivalent change in the excess base, or alkaline reserve, as it is called by chemists. The alkaline reserve may be titrated while boiling the sea water to eliminate CO_2 . The exact value of the titration depends on the indicator used and the exact color of the indicator that is taken as the end point, hence only those titrations done in the same manner can be strictly compared. The titrations, used for the present paper were made by adding di-brom-o-cresol-sulphone-phthalein to 100 cc. of sea water in a flask of resistance glass and titrating with 0.01 *N* HCl, while boiling, until the purple color changed to yellow and did not become purple again after boiling for 5 minutes longer. The sides of the flask must not be allowed to dry as this would cause HCl to escape from the chlorides, due to the action of Magnesium. The results per liter were recorded and some titrations were made at 20° and others at 30°, but the errors due to change in volume of the sea water is within the limits of accuracy of the method. If the sea water is diluted with rain water, the alkaline reserve will be lowered, but this error may be compensated by dividing by the chlorine content (grams chlorine per kilogram sea water). In other words: a change in the quotient of the alkaline reserve by the chlorine per cent indicates a gain or loss of CaCO_3 .

	ALKALINE RESERVE	Cl %.	$\frac{\text{Alk. res.}}{\text{Cl \%}} \times 10,000$
Sea water from San Diego, Cal.....	0.00235	18.7	1.257
Sea water from Woods Hole, Mass.....	0.00240	17.7	1.356
Gulf Stream, off Miami, Fla.....	0.00250	19.9	1.257
Gulf Stream, off Tortugas, Fla.....	0.00250	19.9	1.257
Average, Tortugas, June and July.....	0.00247	20.0	1.235
Average, Key West, June and July.....	0.00237	20.0	1.185

The above table indicates that some CaCO_3 has been removed from Tortugas sea water, as compared with other sea water, and to a greater extent from Key West sea water. In other words, the precipitation observed by Vaughan is not due to a greater amount of calcium in Tortugas or Key West sea water but to local conditions which cause the precipitate to form.

According to the law of mass action, in a saturated solution of CaCO_3 in sea water at constant temperature, salinity, etc.,

$$[\text{Ca}^{++}] \times [\text{CO}_3^{--}] = \text{a constant.}$$

Not all of the calcium is, however, in the form of CaCO_3 and Ca^{++} , for some is undissociated CaCl_2 , CaSO_4 , $\text{Ca}(\text{OH})_2$, and CaHCO_3 . The chlorides and sulphates are constant but $[\text{CaHCO}_3]$ and $[\text{Ca}(\text{OH})_2]$ change with the total CO_2 content of the sea water. But I have shown⁶ that if the alkaline reserve remains constant, the total CO_2 of sea water (within limits found in nature) varies inversely with the pH ($= -\log.H^+$ concentration). Hence the determination of the pH may be substituted for that of the total CO_2 . The determinations I have made of the water of the Pacific and North Atlantic showed the pH to vary from about 8.1 to 8.25 and those of Dr. A. G. Mayer in the Pacific showed only a little wider range (below 8). Earlier observations at Tortugas gave the same range, but my more extended observations made this summer demonstrate the inadequacy of a few determinations. The pH is influenced by plant and animal life and rises at Tortugas to 8.35 during the day over well-lighted bottoms rich in vegetation, and falls to 8.18 during the night. It may be said, therefore, that conditions in shallow water over eel-grass or other sea-weed or corals (with symbiotic algae) favor the precipitation of CaCO_3 .⁷

The question arises whether the occasional high pH of Tortugas sea water is sufficient to explain the precipitation of CaCO_3 , or whether nuclei for the separation of the solid phase are necessary. A large amount of CaCl_2 may be added to sea water without causing a precipitation. If the pH is increased by the addition of NaOH , the result depends on the speed at which the alkali is added. If the NaOH is added suddenly in the form of a strong solution, colloidal precipitation membranes form about the drops and if the membranes are broken by shaking or stirring, a great mass of $\text{Mg}(\text{OH})_2$ is included in the precipitate. If a very dilute solution of NaOH is added very slowly, CaCO_3 , possibly contaminated with $\text{Mg}(\text{OH})_2$, is precipitated. The exact pH at which precipitation first occurs cannot be determined by this method as the first precipitation occurs before the solutions are mixed and the crystals thus formed serve as nuclei for further precipitation. If Tortugas sea water is kept in glass bottles, precipitation occurs on the glass while the pH of the water is within the natural limits, but the pH at the glass surface is higher, due to solution of glass.

Although the pH at which precipitation would occur without nuclei for the separation of the solid phase, may be practically impossible to determine, the final equilibrium with an abundance of nuclei is not a difficult problem. Calcite and aragonite crystals to serve as nuclei were produced by the methods of Johnston, Merwin and Williamson.⁸ The crystals were examined under the microscope and tested with cobalt

nitrate solution. These observations, together with the mode of preparation, leave little doubt that the crystals actually were calcite and aragonite. Under the microscope an occasional calcite crystal could be found among the aragonite crystals but the number was not sufficient to affect the cobalt nitrate test. These calcite crystals apparently grew slightly during the experiments but apparently no new ones were formed. To determine the equilibria, crystals were mechanically stirred or shaken with sea water in 'nonsol' flasks, six to fourteen hours at 30°, then the pH and alkaline reserve determined.

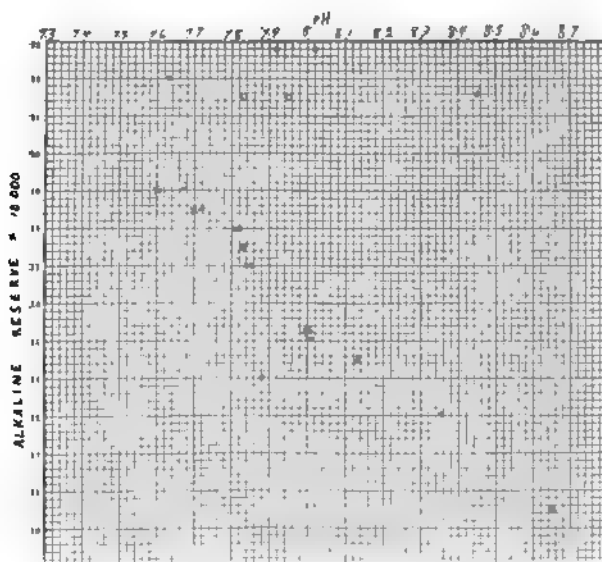


FIG. 1

The results are shown in figure 1. The alkaline reserve is measured on the ordinate and the pH on the abscissa. The results of agitating 10 grams of calcite crystals with a liter of sea water until equilibrium was approximately reached are shown by black rhombs in figure 1. If we draw a straight line from the intersection of the ordinates of pH 7.3 and alkaline reserve 0.0023 to pH 8.8 and alkaline reserve 0.0009, the determinations with calcite will fall very close to it. This shows that sea water of the surface of the oceans of the whole world is supersaturated in respect to calcite. We may therefore conclude that suitable nuclei for the precipitation of calcite are absent or deficient in number. The solubility of crystals varies inversely with their size, but after they have attained sufficient size to be readily examined with low powers of the microscope, further increase has an unappreciable effect on solubility. But such crystals if present, would be rapidly precipitated to the bot-

tom of the sea, hence the absence of nuclei for precipitation of calcite is what one might expect.

Aragonite is said to be about 10% more soluble than calcite, but no difference in the point of equilibrium of the two substances with sea water was detected in these experiments. This may be explained by the facts that a few calcite crystals were mixed with the aragonite, equilibrium was only approximated and there were slight errors in the determinations. The results are shown by black rosettes in figure 1.

During the rough weather, white calcareous mud is stirred with the sea water at Key West and to a lesser extent at Tortugas, and it was thought possible that the mud granules might form nuclei for precipitation and explain the low alkaline reserve at Tortugas and lower alkaline reserve at Key West. On agitating white calcareous mud, dredged from the bottom, with sea water, no definite equilibrium was reached, even at the end of four days. If the alkaline reserve was first lowered by removal of some CaCO_3 , it remained lower than if shaken with calcite and if normal sea water was used the alkaline reserve remained higher than with calcite. It was thought possible that the grains were covered with an impenetrable film of organic matter; so some mud was dried and powdered in a mortar in order to break the pieces and form fresh surfaces, but similar results were obtained with this. The results are shown by black circles in figure 1.

If mud was mixed with an equal weight of calcite, the results were the same as with pure calcite, as shown by the black dumb-bells in figure 1. This mud and calcite on standing in sea water for thirty days had not changed to calcite. All these facts tend to show that the particles in the mud are in some way retarded or prevented from getting into equilibrium with the water.

In order to be sure of clean surfaces of natural calcareous substances, a specimen of coral, *Maeandra clivosa*, was ground and powdered in a mortar and agitated with sea water in the same manner as in previous experiments. The results were similar to those with mud, as shown by the white circles in figure 1.

There seems to be a more soluble form of calcium carbonate (the μ CaCO_3 of Johnston), but since it cannot be obtained in a pure state, no attempt was made to prepare it. One experiment, however, was made with a precipitate of CaCO_3 that appeared as spherical grains under the microscope. It was agitated for twelve hours with sea water and the alkaline reserve was 0.0022 at pH 7.95. No further experiments were made to determine whether equilibrium had been approximated.

These experiments clearly show that the surface water of the sea is a supersaturated solution of CaCO_3 , and it is only necessary to introduce calcite crystals in order to cause considerable precipitation of this substance. Precipitation goes on in the bodies of organisms in the surface waters of all seas. The precipitation observed by Vaughan at Tortugas is very finely divided, but whether it was formed in the bodies of minute organisms, which subsequently died, has not been determined. Such particles might slowly grow, since the agitation of them with sea water was found to take a trace of CaCO_3 out of the water. Small crystals have been seen in the bodies of Protista, and whether they are CaCO_3 , or not, they might form nuclei for the precipitation of CaCO_3 if released into the sea water.

In some experiments in liter flasks of resistance glass, filled into the neck (and hence admitting of but slight loss of CO_2) the pH and alkaline reserve was determined immediately before and after agitation with calcite, and the loss of CO_2 from the sea water calculated from the pH and from the loss of CaCO_3 (alkaline reserve).

SEA WATER	pH	TOTAL CO_2	ALKALINE RESERVE	CALCULATED LOSS OF CO_2	
				From pH	From alk. res.
Before agitation with calcite.....	8.2	44.5	0.0025	6	6.72
After agitation with calcite.....	7.67	38.5	0.0019		
Before agitation with calcite.....	8.25	43.8	0.00250	6.8	7.27
After agitation with calcite.....	7.72	37.0	0.00185		

In the above table, the agreement is very striking in view of the probable error in determination of pH and the liability to loss of CO_2 from the water surface in the neck of the flask, agitated by the rotary stirrer.

If the pH of sea water should be maintained (by the action of plants) at 8.2 while it was agitated with calcite crystals, the loss of CaCO_3 would be about 0.001 N, or 0.0005 M, or 0.1 gram per liter. This would cause a deposit of 10 kgm. per square meter of bottom in water 100 meters deep. This would cause a lowering of the calcium content of Tortugas sea water by about 4.5%.

¹ *The Depths of the Ocean*, London, 1912, p. 178.
² These PROCEEDINGS, 2, 1916, (28).
³ *Washington, Carnegie Inst.*, Pub. No. 182, 1914, (Tortugas Lab., vol. 5).
⁴ *Depths of the Ocean*, p. 368.
⁵ *Voyage of H. M. S. Challenger, Physics and Chemistry*, vol. 1.
⁶ McClendon, *J. Biol. Chem.*, Aug., 1917.

⁷ It would be interesting to know whether corals and calcareous algae deposit as much CaCO_3 in the dark as in the light. Corals from deep water are smaller, more fragile, and deposit less CaCO_3 than those of shallow water, but the same is true of animals without symbiotic algae. The deposition is, however, related to the pH, since Palitzsch has shown that the pH decreases with depth.

⁸ *Amer. J. Sci., New Haven*, **41**, 1916, (473).

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**THE HYDROGEN ION CONCENTRATION OF THE
ILEUM CONTENT**

BY
J. F. McCLENDON, A. SHEDLOV, AND W. THOMSON

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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXI, No. 1, JULY, 1917

THE HYDROGEN ION CONCENTRATION OF THE ILEUM CONTENT.

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(From the Physiological Laboratory of the University of Minnesota Medical School, Minneapolis.)

(Received for publication, May 30, 1917.)

It has been shown by the senior author that the reaction of the duodenal contents of infants is distinctly acid (pH 3.1). A few determinations of the duodenal contents of adults, that had been exposed to air for hours, showed them to have become slightly more alkaline than the blood (pH 7.7) but whether they are alkaline in the body was not determined. One sample of adult duodenal contents that was strongly colored with bile was about as acid as the stomach. According to Foà, dogs' bile and calves' bile is acid (pH 5.4 and 6.5), and it seems probable that the neutralization of the acid from the stomach is accomplished by the pancreatic juice alone. Pancreatic juice is usually mixed with bile in the common bile duct, but the presence of bile in the duodenum may *not always* indicate that pancreatic juice is present also. We opened the abdominal cavity of a dog several hours after a hearty meal and before the stomach was nearly empty. The ileum was empty except for some residue packed in its lower end. The duodenum contained bile-stained fluid of an acid reaction. Auerbach and Pick found the contents of the small intestine of dogs to be slightly alkaline, but it is not clear that some CO₂ was not lost from the samples. Although the exact pH of the small intestines of men and dogs may be uncertain, it is probably higher (less acid) than the duodenal contents of infants. Since we did not have the opportunity to study the contents of the ileum of infants, we used pups, and found the contents of the ileum to be slightly acid throughout the nursing period and later on a diet of solid food. The contents of the ileum were less acid than those of the infants' duodenum (except possibly during the first week), as shown in the following table of seven pups of the same litter.

Age, days....	4	9	11	16	18	42	46
pH of ileum	5.7	6.75	6.34	6.3	6.1	6.15	6.0

The last two pups took only solid food and the acidity of the stomach was as high as in the dog. The younger pups nursed at very frequent intervals; the pH of the stomach contents varied from about 5.5 to 6 and the pH of the ileum was nearly the same as that of the stomach at the same time. The low acidity of the stomach is due to the acid-binding power of the milk.

These experiments offer a suggestion as to the conditions in the infant. The gastric juice of the infant is nearly as acid as that of the adult. The period of 4 hours between feedings allowed the stomach to become empty and to accumulate a very acid fluid which ran down into the duodenum. We suppose such a process would take place in the pup with 4 hour feedings. We have no evidence, however, to indicate whether the reaction of the infant's ileum is the same as or different from the duodenum.

The determinations were made with the hydrogen electrode described by McClendon and Magoon, the tip being inserted through a small hole cut in the ileum and the electrode filled by pressing on the gut. The temperature of the room was electrically regulated to 20°. The Leeds and Northrup potentiometer was the same as in preceding experiments, but a Leeds and Northrup (Type R) galvanometer of about 4,000 ohms was used in place of the capillary electrometer. The oscillations of this galvanometer cause much waste of time, but we damped the oscillations at intervals in the following manner. A key was used that opened the circuit when pressed down half way and closed it when pressed all the way down, but short circuited the galvanometer when released. When the galvanometer is short circuited, the potential energy of the twisted suspension or the kinetic energy of the moving coil is transformed into electrical energy and hence into heat in the highly resistant circuit, and hence the rate of movement of the coil is brought almost to zero.

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THE WAVERLY PRESS
BALTIMORE, U. S. A.

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OF ANIMAL HEAT, AND OF CO₂ OF THE
AIR, WITH A SUMMARY OF SIMI-
LAR DATA ON BICARBONATE
SOLUTIONS IN GENERAL**

BY
J. F. McCLENDON

**(FROM THE PHYSIOLOGICAL LABORATORY OF THE UNIVERSITY OF
MINNESOTA, MINNEAPOLIS)**

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXX, No. 2, JUNE, 1917

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TIONS IN GENERAL.***

By J. F. McCLENDON.

*(From the Physiological Laboratory of the University of Minnesota,
Minneapolis.)*

(Received for publication, March 14, 1917.)

The experiments on which this paper is based were all done by the author, but some of them have already been published (McClendon and Magoon, McClendon, 1916, *b*, and McClendon, Gault, and Mulholland). The main object of the present paper is the extension of this previous work by means of the new and slightly more reliable apparatus herein described. Sea water is much more difficult to investigate than blood, owing to its low buffer value and low CO₂ tension. It requires great care to measure 0.01 mm. in CO₂ tension, and yet this is about 4 per cent of the CO₂ tension of the sea. After the CO₂ tension is determined it may be changed by the solution of glass. The difficulties of making determinations with the hydrogen electrode are increased as the buffer value is lowered.

A Leeds and Northrup potentiometer and a 0.1 N KCl calomel electrode were used.¹ The mercury was redistilled in a Hulett still. The hydrogen electrode is shown in Fig. 1 as set up for

* Some of the apparatus was bought with a grant from the research fund of the Graduate School, and some was borrowed from the Marine Laboratory of the Carnegie Institution.

¹ Electrolytic calomel for this was kindly sent me by Professor G. A. Hulett.

the passage of the H₂ + CO₂ mixture through it. It was made of a tube of 24 mm. bore with a stop-cock at each end and a gold disk welded to a platinum wire fused through the glass. The gold disk was covered with palladium black by electrolyzing a 1 per cent filtered solution of palladium chloride, using a small platinum wire as anode. A 4 volt current was used, but this had to be cut down by reducing the size of the anode, especially at the beginning. If the palladiumization proceeds too fast, the palladium black does not stick to the cathode, but breaks off and moves to the anode in a cloud. After the electrode had been used a few times the black was dissolved off by concentrated nitric acid, and deposited anew.²

The gas mixture was admitted into the electrode by a swivel joint ground so true that it could be effectively closed with a water seal. The electrode was rotated 400 revolutions per minute by means of a Tiffany motor operating on a cork wheel or pulley. If the electrode contained only 2 cc. of sea water and its CO₂ tension at the start did not differ from that of the gas mixture more than 0.1 mm., equilibrium was reached by the passage of a liter of the gas mixture in the course of 30 to 40 minutes.

The gas mixer is also shown in Fig. 1 and is of 1 liter capacity, with the upper portion graduated and so narrow that 0.00001 liter can be read on it with ease. The 3-way stop-cock at the top allows the connecting tubes to be washed out with the gas to be introduced. Since the apparatus holds 25 pounds of mercury, the gas mixer and leveling bulb were wound with iron wire

² If the nitric acid is contaminated with chlorides or HCl, chlorine will be formed. If it is necessary to remove platinum black from electrodes, aqua regia is required and an abundance of chlorine is formed. The last trace of chlorine may be removed by electrolyzing a dilute solution of H₂SO₄, using the electrode as cathode. If, however, it is feared that a palladiumized electrode is contaminated with chlorine, it is safest to electrolyze distilled water with a higher voltage, as palladium black is attacked by mineral acids. The fact that palladium black is attacked by HCl may explain the rapid deterioration of palladiumized electrodes in gastric juice, which I have repeatedly observed. Another objection to palladium black is that it amalgamates instantaneously with mercury. The surface of hydrogen electrodes is "poisoned" by so many different substances that it often seems impossible to find the cause of the trouble. Both oxidizing and reducing gases may poison it.

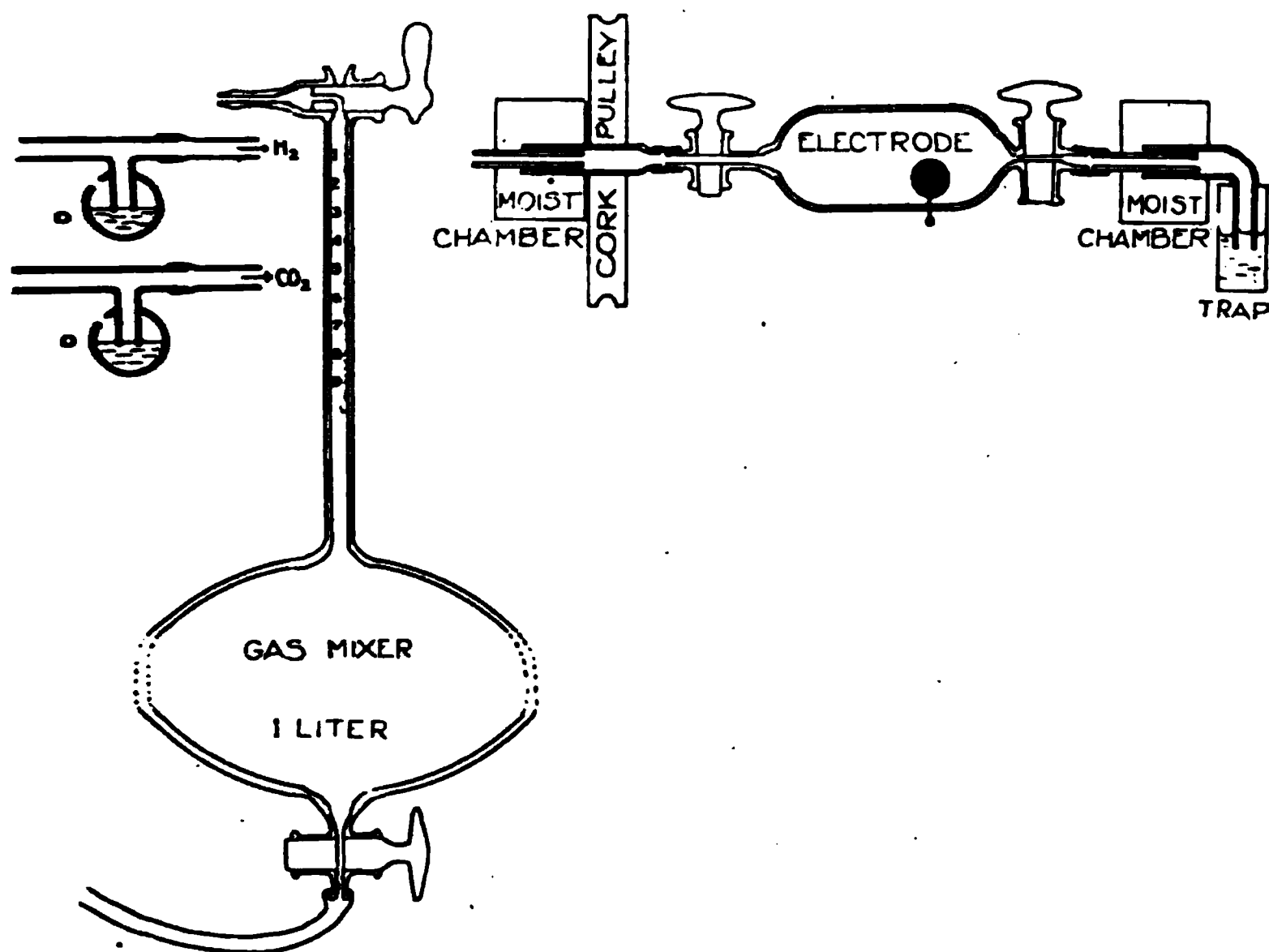


FIG. 1. Gas mixer and rotating electrode for determining the pH of a solution at any desired CO_2 tension. The gas mixer at the left is of 1 liter capacity down to the mark just above the lower stop-cock, and the numbers on the upper narrow portion mark 0.0001 liter. The mixer is filled with mercury and the CO_2 generator connected through the trap that delivers CO_2 at atmospheric pressure, to the upper stop-cock, which is first turned so that the gas escapes at the top and later reversed so as to admit the desired amount of gas. These operations are repeated with H_2 and the mixer is filled with it down to the 1 liter mark at the bottom. The remaining mercury is shaken with the gas to mix it. The mixer is now turned around and connected with the rotating electrode on the right through the swivel joint closed by a water seal in the moist chamber. A similar water seal connects the right hand end of the electrode with a trap to prevent the backward diffusion of air. The electrode (containing 1 or more cc. of the solution) is rotated 400 revolutions per minute by means of the cork pulley belted to a Tiffany motor while the gas mixture is slowly passed through it by displacement with mercury in the mixer. The stop-cocks at the two ends of the electrode are closed, and the one nearest the palladiumized disk (having been left ungreased) is immersed in the KCl bath and a wire hooked in the projecting loop from the palladiumized disk. The reading is now taken in the usual manner.

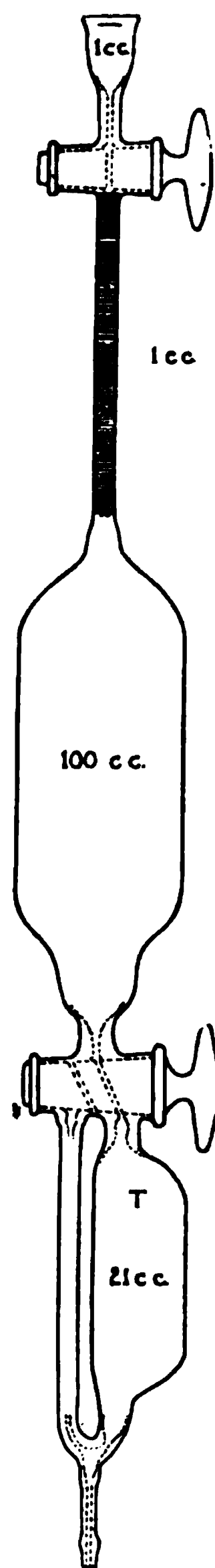


FIG. 2.

FIG. 2. Van Slyke apparatus slightly modified for determining the total CO_2 in sea water. A rubber suction tube, 1 mm. bore and 1 meter long, connects the lower end with a leveling bulb with cylindrical sides and filled with mercury. By raising the bulb the apparatus is filled with mercury. The upper stop-cock is closed and the bulb lowered so as to exhaust the apparatus. The bulb is raised without opening the upper stop-cock. The traces of air are now collected at the top and are forced out on opening the upper stop-cock. By means of careful manipulation of a pipette and the upper stop-cock, 10, 15, or 20 cc. of sea water are admitted, care being taken not to admit any air. It is permissible to leave one or two drops of this sea water in the 1 cc. cup at the top as this amount may be washed down by the introduction of 1 cc. of 2 N HCl in the same manner. A drop of mercury is placed in the cup to seal the stop-cock and the bulb lowered until the mercury falls to the etched mark near the bottom of the 100 cc. chamber. The lower stop-cock is closed and the apparatus is shaken laterally in the vertical position as vigorously as possible for 2 minutes (in case 20 cc. of sea water was used, 4 minutes). The sea water is now trapped off in the trap, T, and the mercury allowed to rise in the 100 cc. compartment until atmospheric pressure is attained in it and the lower stop-cock closed. At the end of 1 minute the amount of sea water above the mercury in the 1 cc. gas burette is measured, calculation being made for the two menisci. The apparatus is laid on its left side on a cushion and agitated 1 minute after the air is in the wide portion of the 100 cc. chamber. The apparatus is then clamped upright, the lower stop-cock opened, and the mercury levels are adjusted by means of a screw. The air volume in the 1 cc. burette is carefully measured. 1 cc. of 0.5 to 1.0 N NaOH is admitted from the cup into the gas burette to absorb the CO_2 . In leveling the bulb to measure the gas residue, one-thirteenth of the height of the NaOH is reckoned as mercury. The total CO_2 that was in the original sea water is that absorbed plus that in the sea water above the mercury plus that in the sea water trapped in the trap, T. After these are calculated by means of the volumes, absorption coefficient, and CO_2 tension, they are added together and reduced to 0° and 760 mm. To do this it is necessary to know the barometric pressure and the capillary depression in the gas burette, which latter is subtracted from the former.

In the following example on 10 cc. of 0.576 N sea water at 20° , the absorption coefficient for CO_2 was 0.757, the barometric pressure 754 mm., and the capillary depression 2 mm. The CO_2 absorbed was 0.405 cc. and the gas volume before absorption 0.59 cc., making the CO_2 tension 0.687 (unity = barometric pressure). The sea water above the mercury was read as 0.075, to which was added the volume of menisci (0.0133) making 0.0883. The CO_2 in this sea water was $0.0883 \times 0.687 \times 0.757 = 0.0459$, which added to the absorbed CO_2 makes 0.4509. This occupied a space of 89 cc. over 11 cc. of acidulated sea water; hence there is in the trap $\frac{11}{89} \times 0.4509 \times 0.757 = 0.0422$, which, added to that already calculated, makes a total of 0.4931 cc., and which, reduced to 0° and 760 mm., is 0.442 per 10 cc., or 44.2 cc. per liter. Since the pH was 8.2 and the alkaline reserve 25, the total CO_2 agrees with Fig. 4.

that was run through boiling sealing wax as it was wound on. After the gases were measured into the mixer, they were thoroughly mixed by shaking up the little mercury remaining in it.

The hydrogen was generated from zinc and H₂SO₄ and passed through a large and a small wash bottle of HgCl₂ solution, and a small one of NaOH solution and one of H₂O. When the small wash bottle of HgCl₂ showed the first trace of discoloration with arsene, all the wash bottles were refilled. The CO₂ was generated from marble and HCl and washed with NaHCO₃ and H₂O. The gases were led to the gas mixer through traps (0, 0) that delivered them at atmospheric pressure, and a barometer in the same room was read during each experiment.

When a liter of the gas mixture had been passed through the revolving electrode, the stop-cocks were closed. The ungreased stop-cock was immersed in the usual KCl bath, a wire hooked into the platinum loop, and the electric potential determined. An identical gas mixture was made and passed through the electrode as before. If the potential remained the same, it was assumed that equilibrium with the gas mixture had been reached. In case it was desired to determine the total CO₂ on the same sample it was necessary to have 10 cc. of sea water in the electrode. The total CO₂ was determined by means of a modified Van Slyke apparatus for determining the CO₂ in serum (Fig. 2).

All the determinations were made in a constant temperature room automatically controlled within 0.2° by means of the apparatus previously described (McClendon, 1916, a). A damping vane in glycerol was added to the bimetallic thermoregulator. Some trouble was caused by arcing between the relay contacts carrying the 1,500 watt heating current. A lump of solder was placed on them so that it fell and rang a bell before a hot arc had time to form. Since a man's body heats 1 cubic meter of air about 0.5° per minute, rapid stirring of the air is needed. This was accomplished by means of a one-sixth horse power electric fan and two smaller fans. As the outdoor temperature varied from 0° to -30°, no special arrangement for cooling the room was required, but the electric heat regulation was later found sufficient even with a window slightly raised and a vertical fan set in front of it.

Great care had to be taken in the manipulation of the Van Slyke apparatus and the reading of the menisci in order to reduce the error below 1 per cent. The greatest difficulty was experienced in greasing the stop-cocks so that they would hold a vacuum without soiling the interior of the apparatus with grease, but this difficulty disappeared on reducing the bore of the stop-cocks to 1 mm. A mixture of equal parts of soft paraffin, vaseline, and chicle, melted together and thoroughly stirred was found to be the best stop-cock grease. It was thinned with vaseline for lower temperatures. A source of error, the magnitude of which has not been determined, is the holding of some CO_2 by the thin film of sea water between the mercury and the glass, when the mercury is readmitted into the large compartment. This source of error is smaller in the modified apparatus. This error makes the values obtained too low, but in the standardization of the apparatus with Na_2CO_3 solution in CO_2 -free distilled water, this error was so small as to be overcompensated by the absorption of CO_2 from the air by the solution in introducing it into the apparatus. In the case of sea water, some CO_2 is rarely lost and never gained.

The titration alkalinity or alkaline reserve was determined by titrating 100 cc. of sea water while boiling in an Erlenmeyer flask of resistance glass with 0.01 N HCl, using dibrom-*o*-cresol-sulfophthalein as indicator. It usually required 1 cc. more than by the usual method of titration with phenolphthalein. The latter indicator was discarded since it was thought to be affected by some of the weak, non-volatile acids in sea water, although not by boric acid. The 0.01 N HCl is affected by the solution of glass about ten times as rapidly as 0.1 N acid (being noticeably changed in a month), and hence the stock solution was made of the latter and the former made from time to time by dilution. If a liberal supply of sea water is at hand, it is very desirable to make the titration on a liter of it with 0.1 N acid, as the end-point is not very sharp in any case. The water should remain yellow after vigorous boiling for 5 minutes after the last acid has been added.

In the experiments on which this paper is based, all liquid volumes were determined at 20° and the solutions used at 10°, 20°, and 30° without correction for volume change. The density

at 20° compared with distilled water at 4° is given. The concentration of the sea water is indicated by the normality of the chloride titration with silver nitrate and may be reduced to gm. per liter by multiplying by 35.46 and gm. per kilo by dividing the product by the density. The alkaline reserve is indicated by the number of cc. of 0.1 N HCl required to titrate a liter of the sea water. The total CO₂ is expressed as the number of cc. of dry CO₂ at 0° and 760 mm. that may be evolved from a liter of sea water by adding acid and boiling (but as stated above, the results are within the limits of accuracy of the micro method). The CO₂ tension is expressed as mm. of mercury, and may be reduced to atmospheres by dividing by 760. The pH was determined by the hydrogen electrode at the same temperature at which the CO₂ tension was regulated, but it was also found that the pH is not perceptibly affected by temperature provided there is no gain or loss of CO₂. The temperature change in hydrolysis of alkaline carbonates is compensated by the change in the dissociation of water.

Besides the Tortugas sea water previously investigated, determinations were made on 0.5366 N sea water from near San Diego¹ (alkaline reserve = 23.5, determined density = 1.0238), on 0.513 N sea water from Woods Hole, Massachusetts (alkaline reserve = 24, determined density = 1.0225), and especially on 0.576 N sea water (alkaline reserve = 25, determined density = 1.0254) taken from the Gulf Stream off Miami, Florida, and examined immediately on arrival by express. No difference was detected between these sea waters in the relation of CO₂ tension to pH. The relation of the total CO₂ to pH was affected only by the alkaline reserve (within the limit of error of the micro method).

It was found that the pH plotted against the logarithm of the CO₂ tension made a very gentle curve at higher CO₂ tensions. Within the limits given in Fig. 3 it was indistinguishable from a straight line (*i.e.*, the curvature is within the limits of error of the determinations). If the CO₂ tension remains constant the pH varies directly with temperature, 1° corresponding to 0.01

¹ This was sent by the Scripps Institution of Biological Research. The water from Woods Hole was sent by Professor A. W. Johnston, and that from Florida was taken by John Mills of the Carnegie Marine Laboratory, and sent by Dr. Alfred G. Mayer.

pH. Fig. 3 may be used as a conversion table for finding the CO_2 tension of sea water from the pH and temperature. These values compare favorably with those of Henderson and Cohn in so far as comparison may be made.

. When the pH is plotted against the total CO_2 , the curvature is possibly greater, but the limits of error are greater, and the graph shown in Fig. 4 is as accurate as it has been possible to make from the data so far accumulated. This figure may be used as a conversion table for finding the total CO_2 from the pH and the alkaline reserve. The lines are not straight if extended. In one determination at pH 7.4 and alkaline reserve 25, the total CO_2 was 54 cc., whereas by the extended chart it would be 57 cc.

Since there is from twenty to thirty times as much CO_2 in the sea as in the air, the small surface of contact of these two cannot locally affect the CO_2 content of the sea water very much. The oxygen content of the sea water is more significantly affected, since it varies with the climatic zones, but the exchange of O_2 between sea and air is probably very slow. To the extent that the sea is a closed system, O_2 varies inversely to CO_2 , due to the action of organisms, the possible error being 30 per cent. We may therefore use Fig. 4 for finding the oxygen content of sea water, provided the pH and alkaline reserve are known, and on the assumption that the respiratory quotient is unity and that a kilo calorie will raise the temperature of a liter of sea water 1° . It is obviously impossible to use these data in any attempt to determine the respiratory quotient of a marine animal in a sealed jar of sea water, but the data are valuable in indicating the limits of the oxygen supply, since the respiratory quotient of animals has been found to vary within narrow limits (0.7 to 1.0). The animal heat per cc. of CO_2 produced by the burning of carbohydrates is about 5 gm. calories, of proteins about 5.9, and of fats about 6.6. The error in estimating the total animal heat with the aid of Fig. 4 will be great only in case the respiratory quotient varies greatly from unity; *i.e.*, when a large proportion of fats and proteins is burned. It seems to be a fact that no gill-breathing animal has a temperature more than $2-3^\circ$ above the surrounding water. After an inspection of Fig. 4 it seems incredible that even the recorded temperatures of aquatic animals could be maintained. The oxygen necessary for the generation

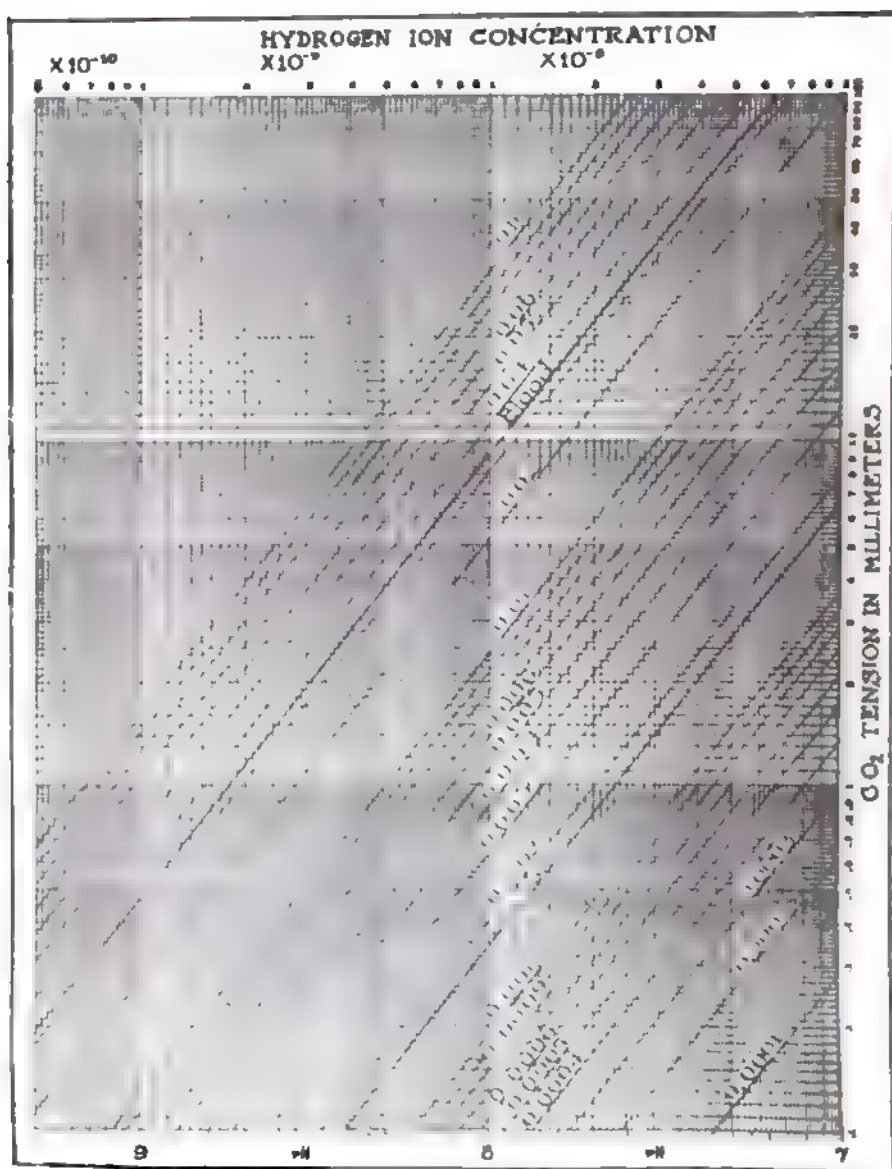


FIG. 3. Conversion table for finding the CO₂ tension at 20° of bicarbonate solutions, sea water, and normal blood from the pH. A simple calculation may be made for any other temperature since it is

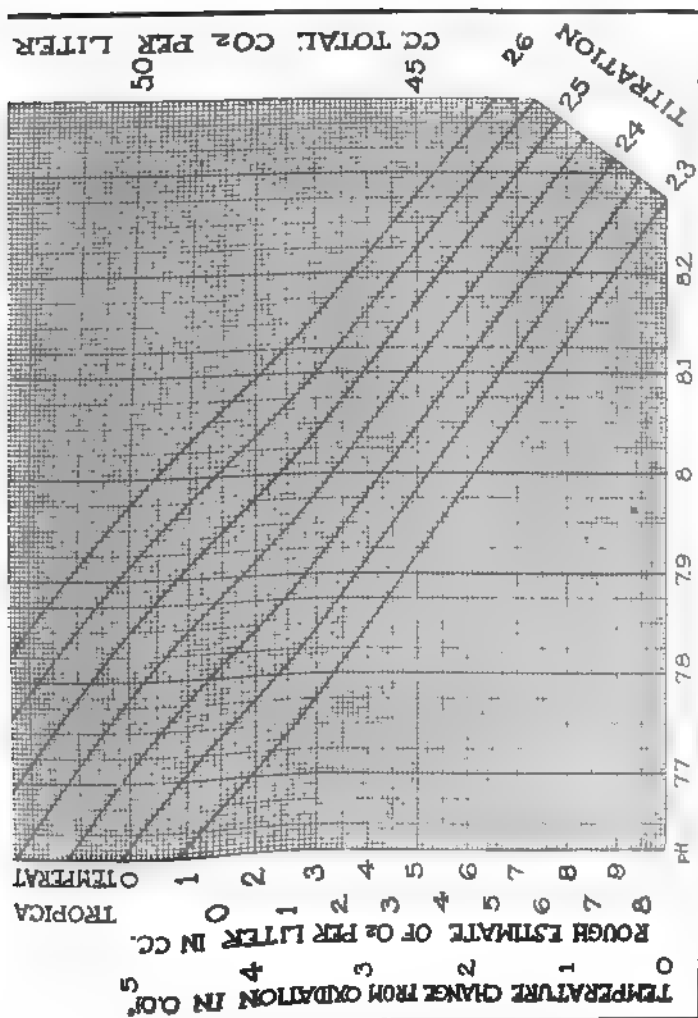


FIG. 4. Conversion table for finding the total CO₂ of sea water from the pH and alkaline reserve (cc. of 0.1 N HCl to titrate 1 liter of boiling sea water). The total CO₂ is measured on the ordinates and the pH on the abscissae. Each diagonal is for sea water of the alkaline reserve indicated. At the left is given the expected O₂ per liter for tropical and again for temperate oceans. If the change in CO₂ is due to animal oxidations, the temperature change caused thereby is indicated in hundredths of a degree.

of heat must be carried from the gills to the interior by an aqueous fluid of high specific heat, and it would appear that the heat transfer must be nearly as effective as the oxygen transfer. The possession of hemoglobin, thus facilitating the oxygen transfer, should, however, allow a slightly higher body temperature to be maintained. A parallel case is indicated by Dallwig, Kolls, and Loevenhart in the comparison of the oxygen necessary to support a flame and maintain the temperature of a mammal. The flame can be maintained in 20 mm. O₂ tension made by rarefying the air, because the decreased oxygen content of the air is compensated by the decreased conduction of heat. But the flame is extinguished at 116 mm. O₂ tension made by diluting the air with nitrogen, because its specific heat is maintained. The mammal is unaffected by either process down to 40 mm. O₂ tension and perhaps farther, because the heat conductance depends largely on the specific heat of the blood, which is constant.

It follows from Figs. 3 and 4 that the total CO₂ plotted against the logarithm of the CO₂ tension forms a straight line. If Fox's data are plotted in this way, a straight line is approximated only for low CO₂ tensions. He used 500 cc. samples and hence his determinations were probably far more accurate than mine. He determined the CO₂ tension by titrating the CO₂ in air shaken with the sea water (and the sea water left in the bottle) by the Pettenkofer method. Perhaps the limits of error of this method could include the differences between Fox's data and mine. Or perhaps the air Fox analyzed had not come to equilibrium with the sea water. Fox discarded whole series of his data, and the remaining closely followed an empirical formula. I am not attempting to overthrow the determinations Fox made with his elaborate gas analysis apparatus by means of a micro method designed for other purposes than that for which it was used. I can only say that the two sets of data are for the present uncertain. It may be significant that his pH data are unreconcilable with mine. He determined these with the hydrogen electrode and also calculated them from the law of mass action and found the pH of sea water to be about 6, whereas I find it to be about 8. In this my results are in harmony with those of Palitzsch, Henderson and Cohn, and others.

Since Figs. 3 and 4 may be used as conversion tables provided the two titrations are made and the temperature and pH are determined, it seems desirable that a method be perfected for determining the pH on shipboard or in poorly equipped laboratories. The colorimetric method is the only one adaptable, and phenolphthalein is objectionable because errors in concentration simulate changes in pH. The buffer mixtures in use have a very different salt concentration from sea water, and the salt error has to be determined for every new indicator. For these reasons it seemed desirable to use the new indicators recommended by Lubs and Clark with buffer mixtures having the same salt concentration as sea water. Sea water varies in concentration, but the range from 0.4 to 0.6 N is perhaps great enough for all purposes. It was also desirable to increase the concentration of the buffers so that they will be less affected by the solution of glass. Since the solubility of buffers is reduced by the increase in salt concentration, the latter was made to correspond to 0.4 N sea water and a slight correction applied for 0.5 N sea water and a greater one for 0.6 N sea water. In actual practice the correction for 0.5 N sea water has already been made on the labels on the sealed tubes of buffers. A correction of 0.05 pH is then indicated for 0.6 N sea water and a similar correction but in the reverse direction for 0.4 N sea water. The salt error between the buffer mixtures and 0.5 N sea water was redetermined with the hydrogen electrode a large number of times at different temperatures and for both thymolsulfophthalein and *o*-cresolsulfophthalein, and likewise with 0.6 N sea water, but I do not believe this absolutely necessary. A moderate dilution of sea water with distilled water does not appreciably change the pH if the CO_2 tension is near that of the atmosphere and it is not agitated with or exposed to the air. It is then only necessary to dilute the sea water to determine the salt error with any new indicator, taking the 0.4 N sea water as the standard for the calculation of the correction in the pH of 0.5 and 0.6 N sea water. The salt error for the above indicators holds for phenolsulfophthalein and probably for all sulfophthalein indicators.

The buffer mixtures are made from two stock solutions "boric" and "borax," kept in Squibb's automatic burettes provided with soda-lime tubes. The distilled water used in making them is

boiled 15 minutes to rid it of CO₂ and cooled in a tightly stoppered, narrow necked flask or by means of a stream of CO₂-free air. The boric acid is recrystallized and dried in a desiccator (not by heat). The borax is recrystallized and dried in dry air short of efflorescence. The NaCl should be pure, but drying by heat is unnecessary because the error due to occluded moisture in the crystals is too small to cause a noticeable difference in the color of the indicators.

The "boric" contains 18.6 gm. of boric acid and 22.5 gm. of NaCl to the liter.

The "borax" contains 28.67 gm. of borax and 19 gm. of NaCl to the liter, and has the same salt action as the "boric" on the indicators. If kept at a low temperature, borax crystallizes out and must be made uniform in solution before mixtures are made. The same applies to the mixtures. The desired indicator is added to the stock solutions to the extent of 10 mg. to the liter, or added to the mixtures in the same proportion. In order to avoid dilution of the buffers and also to avoid the necessity of weighing the indicator for each solution, it was made up in 0.1 per cent solution in alcohol redistilled over sodium. Thymol-sulfophthalein requires the addition of a little NaOH to get it into solution at this concentration and hence the solution becomes less sensitive if allowed to absorb CO₂. If 0.01 per cent aqueous solution of the indicator is used for addition to sea water, the concentration of the sea water after the addition is used in calculating the salt error, since this dilution of 10 per cent corresponds to 0.025 pH in the salt error.

30 cc. of each mixture containing the indicator were sealed up (by fusing the glass) in a "Nonsol" test-tube of exactly 24 mm. bore without introducing CO₂ from the blast lamp. Since these indicators are impure, it is necessary to test each lot, both as to concentration and range. The concentration may be tested by comparison with a sealed tube of the original indicator in distilled water, the concentration being the same as in the mixtures. In order to test the range, it is necessary to save samples of the stock solutions without indicator. This may be done by making mixtures, one near the middle or one near each end of the range of the indicator and sealing them in "Nonsol" tubes. It is then only necessary to cut off the tip of the seal and introduce the

new sample of indicator in order to test it. The "boric" allows the growth of mold, but the "borax" is antiseptic. The tubes containing a large proportion of "boric" should be sterilized by immersion in water up to the air space and boiling the water.

To facilitate comparison of the tubes, a colorimeter was made by placing stereoscope prisms together in a sharp line and placing two of the tubes at such a distance behind them that the centers of the images were brought together in a sharp line. A thin milk-white (opal) glass was placed immediately behind the tubes to disperse the direct sunlight or mazda light passed through "daylite" glass.⁴

The mixtures are given in the table on the following page.

The salt action on the indicators is approximately directly proportional to the salt concentration over the range from 0.1 to 0.6 N, the difference in salt error of two sea waters being one-half to three-fifths the difference in normality, and increase in salinity causing the same color change as increase in alkalinity (increase in pH).

The useful range of *o*-cresolsulfophthalein is from the first of the series to pH 8.3 and of thymolsulfophthalein from 7.9 to the last of the series. For the surface water of the open sea one indicator is about as good as the other, except in the tropics, where thymolsulfophthalein is the best. In the study of the respiration of marine animals *o*-cresolsulfophthalein or α -naphtholsulfophthalein should be used.

A more or less definite relation between the pH and the solubility of calcium salts in the sea water seems to exist. Dittmar showed that there is less calcium in the surface waters than in the deep waters of the ocean, and Sørensen and Palitzsch showed that the pH is higher in the surface waters. It is difficult to study this question *in vitro*, owing to the relative stability of the supersaturated solutions of CaCO_3 , and the existence of it in solid form as aragonite, calcite, lublinitite, and vaterite, with different solubilities. Presumably the surface water over lime mud flats in the tropics is saturated with calcite, or nearly so. If CaCl_2 is added to this water, in the form of a concentrated solution, no precipitate occurs, but if the pH is only slightly increased, CaCO_2 begins to deposit on the glass, and it takes relatively little increase in the pH to cause a precipitate

⁴ The sealed tubes and colorimeter may be obtained from Hynson, Westcott and Dunning, Baltimore. The tubes are labeled for 0.5 N sea water.

280 pH and CO₂ Tension of Bicarbonates

"Boric."	"Borax."	pH of sea water.			"Boric."	"Borax."
		0.4 N; all in- dicators.	0.5 N; sulfo- phthalein.	0.6 N; sulfo- phthalein.		
<i>per cent</i>	<i>per cent</i>				<i>cc.</i>	<i>cc.</i>
79.5	20.5	7.50	7.45	7.40	23.90	6.1
78	22	7.55	7.50	7.45	23.4	6.6
76	24	7.60	7.55	7.50	22.8	7.2
74	26	7.65	7.60	7.55	22.2	7.8
72	28	7.70	7.65	7.60	21.6	8.4
70	30	7.75	7.70	7.65	21.0	9.0
68	32	7.80	7.75	7.70	20.4	9.6
66	34	7.85	7.80	7.75	19.8	10.2
64	36	7.90	7.85	7.80	19.2	10.8
62	38	7.95	7.90	7.85	18.6	11.4
60	40	8.00	7.95	7.90	18.0	12.0
58	42	8.05	8.00	7.95	17.4	12.6
56	44	8.10	8.05	8.00	16.8	13.2
54	46	8.15	8.10	8.05	16.2	13.8
52	48	8.20	8.15	8.10	15.6	14.4
51	49	8.22	8.17	8.12	15.3	14.7
49.5	50.5	8.25	8.20	8.15	14.85	15.15
47	53	8.30	8.25	8.20	14.10	15.9
44.5	55.5	8.35	8.30	8.25	13.45	17.55
42	58	8.40	8.35	8.30	12.60	17.4
39.5	60.5	8.45	8.40	8.35	11.85	18.15
37	63	8.50	8.45	8.40	11.10	18.9
34.5	65.5	8.55	8.50	8.45	10.35	19.65
32	68	8.60	8.55	8.50	9.6	20.4
29	71	8.65	8.60	8.55	8.7	21.3
26	74	8.70	8.65	8.60	7.8	22.2
23	77	8.75	8.70	8.65	6.9	23.1
20	80	8.80	8.75	8.70	6.0	24.0
17	83	8.85	8.80	8.75	5.1	24.9
14	86	8.90	8.85	8.80	4.2	25.8
11	89	8.95	8.90	8.85	3.3	26.7
8	92	9.00	8.95	8.90	2.4	27.6
4.5	95.5	9.05	9.00	8.95	1.35	28.65
1	99	9.10	9.05	9.00	3.0	29.7

throughout the solution. The size of grain makes a difference in the solubility of the precipitate, but if time for equilibrium is allowed, the small grains will change into less soluble crystals. According to Irvine and Young, sea water will dissolve 125 parts per million of crystallized CaCO₃. Rona and Takahashi determined the total Ca and the pH in mixtures of Na and Ca carbonates, bicarbonates, and chlorides, in contact with pro

precipitated CaCO_3 . The concentration of the chlorides varied, but the alkaline reserve was about 160 in each. The following table gives the pH and the normality of $\frac{1}{2}$ Ca.

pH.....	6.65	6.80	6.89	6.97	7.03
Ca N.....	0.00985	0.0064	0.00492	0.00455	0.00405

In sea water the pH is about 8, the alkaline reserve is about 25, and the normality of $\frac{1}{2}$ Ca about 0.1. The greater solubility of Ca in sea water notwithstanding the greater pH is due to decreased concentration of total CO_2 and possibly to the increased concentration of chlorides. Since the chlorides are nearly constant and the alkaline reserve, total CO_2 , and Ca are interdependent, any change in the pH must cause a change in the solubility of Ca. When the pH is sufficiently raised, CaCO_3 is precipitated and the Ca content and alkaline reserve are lowered. At present the law of mass action cannot be applied to such complex mixtures containing divalent salts whose second dissociation constant is unknown. Harkins and Harce found that the addition of a salt with a common polyvalent ion may increase rather than decrease the solubility of a polyvalent salt. It is possible that the presence of CaCl_2 may increase rather than decrease the solubility of CaCO_3 in sea water. The solubility product law as applied to univalent salts does not apply without modification to polyvalent salts of certain types.

In a previous paper (McClendon, 1916, *b*) I described an experiment in which the CO_2 tension of the air was determined by drawing it through sea water and then determining the pH of the sea water. A considerable time was required to reach equilibrium, and therefore special precautions had to be taken to prevent contamination or evaporation of the sea water. In order to avoid these precautions, I have experimented with bicarbonate solutions of such low alkaline reserve that equilibrium was reached quickly. In the meantime Higgins and Marriott published similar experiments, but their method was not sensitive enough for my purposes. I found that the pH of a 0.0003 N NaHCO_3 solution as measured colorimetrically with phenolphthalein and Sørensen's phosphate mixtures changed with CO_2 tensions as shown in Fig. 3, for 20° . The pH varied directly with the temperature, 1° corresponding to 0.01 pH. Hence the 0.0003 N line in Fig. 3 may be used as a conversion table to find the CO_2 tension of the air from the pH and temperature. If the temperature is 19° , 0.01 is added to the pH before using the conversion table, and if the temperature is 27° , 0.07 is subtracted from the pH before using the conversion table.

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The phenolsulfophthalein was made up in 0.04 per cent solution in absolute alcohol and 5 cc. of it were added to 300 cc. of the phosphate solutions. The latter, M/15 solutions of KH₂PO₄ and Na₂HPO₄, were mixed in the following proportions:

pH	Na ₂ HPO ₄	KH ₂ PO ₄	pH	Na ₂ HPO ₄	KH ₂ PO ₄
7.00	18.2	11.8	7.40	24.2	5.8
7.05	19.0	11.0	7.45	24.7	5.3
7.10	19.8	10.2	7.50	25.2	4.8
7.15	20.6	9.4	7.55	25.6	4.4
7.20	21.4	8.6	7.60	26.0	4.0
7.25	22.2	7.8	7.65	26.4	3.6
7.30	23.0	7.0	7.70	26.7	3.3
7.35	23.6	6.4			

These mixtures were sealed in "Nonsol" test-tubes of exactly 24 mm. bore. The apparatus for testing the air was shaped similar to a Dutchman's pipe. The bowl consisted of a short piece of "Nonsol" test-tube of exactly 24 mm. bore, and the stem of a piece of "Nonsol" tubing of about 7 mm. bore and a meter long. About 10 cc. of 0.0003 N NaHCO₃ solution, containing the same concentration of phenolsulfophthalein as the phosphate mixtures contain, was placed in the bowl of the pipe and suction applied to the mouthpiece by means of the mouth of the operator, so that the air passed up through the solution in bubbles in the pipe-stem. This process was continued until no further color change occurred in the solution, which was then allowed to run back into the bowl and its color compared with that of the sealed tubes. A thermometer was then placed in the bowl and the temperature of the solution determined.

By making the bowl of the pipe 10 mm. bore, it was found possible to use Hynson and Westcott phenolsulfophthalein tubes made for determination of pH of blood dialysate, but with less accuracy.

The main difficulty experienced has been in keeping a 0.0003 N solution of NaHCO₃. A gold flask would be too expensive and a pure silver flask hard to procure. It seems unwise to invest in a gold-plated flask without the guarantee that the plate will remain perfect. Ceresin bottles may be used, but ceresin and

rubber lacquer peeled off. I silvered the interior of a flask of resistance glass. The silvering peeled off in the upper part of the neck, and was affected by sulfur from the rubber stopper. If gold is burned on the glass in a layer thick enough to be opaque, it peels off. If the layer is thinner, it may stick for a long time. I gilded one resistance flask as follows: Campana's bright liquid gold was thinned with banana oil and poured into the flask and poured out, and dried thoroughly in an inverted position by means of a current of air. It was then heated in the blast lamp. Perhaps better results might be obtained by substituting regular thinner made for china painter's gold, and a china kiln. Irving Langmuir has described⁵ a process of gilding by volatilizing gold on an electrically heated tungsten filament in high vacua. Ceresin bottles do not absorb the indicator and are perhaps the best for holding the bicarbonate solution colored with the indicator. The indicator undergoes some change in absolute alcohol.

The time required for reaching equilibrium is lessened if air is passed through the stock solution of bicarbonate so as to remove some of the CO_2 . The stock solution may be made by passing CO_2 or the breath through a NaOH or Na_2CO_3 solution. The more CO_2 the solution contains, the less it attacks glass, silica, and other flask materials, and rubber stoppers. Sulfur-free rubber stoppers are very desirable for closing the flasks. On a trip from San Francisco to Samoa and return, Dr. A. G. Mayer found the CO_2 tension of air over the Pacific to vary from about 0.23 to 0.3 mm. by this method, whereas the variation of the CO_2 tension of the sea surface was much greater and not correlated with that of the air. This indicates that diffusion of CO_2 between air and sea is very slow.

When the CO_2 tension is known, the per cent of CO_2 in the air may be found by multiplying by 100 and dividing by the barometric pressure in mm.

In the determination of the CO_2 of the air and in other studies, number of NaHCO_3 solutions were investigated, as shown in g. 3. These were preliminary determinations, and no special accuracy is claimed for them. The chief source of error probably lies in failure to reach equilibrium, as shown by the follow-

⁵ Paper presented before the American Physical Society, 1916.

ing example on sea water. A rapid stream of CO₂-free hydrogen was passed through a few cc. of sea water and at the end of 27 minutes the alkali had been reduced to carbonate, but only a small fraction to NaOH, and yet CO₂ was being continuously eliminated at the end of the experiment. The experiments were accurate enough, however, to indicate, at least within certain limits, that at constant temperature the pH of these solutions is proportional to the logarithm of the alkaline reserve and inversely proportional to the logarithm of the CO₂ tension. If the CO₂ tension remains constant, a rise of 1° in temperature causes an increase of 0.01 pH, whereas if the total CO₂ content remains constant, the pH is not affected by temperature. At constant CO₂ tension and temperature, as shown by Fox, the total CO₂ is directly proportional to the alkaline reserve within a slight error equal to the CO₂ absorbed by distilled water under the same conditions.

My experiments indicate, at least within certain limits, that the slope of the curves, as in Fig. 3, is the same for sea water, blood, and other biological media (except those exceptionally rich in phosphates) as it is for bicarbonate solutions. Neutral salts slightly decrease the hydrolysis of bicarbonate and decrease the pH. The chief buffer in these media is bicarbonate, and other buffers are not present, even in blood, in sufficient concentration to change the slope of the curve greatly, at least in its upper regions, but the buffer action of proteins in blood comes into play when the blood is made strongly alkaline by the elimination of CO₂, the curve for blood in Fig. 3 being slightly incorrect in the lower CO₂ tensions.

It would be of interest in this connection to know the concentration of bicarbonate in normal blood, but the presence of phosphates and organic matter makes titration very uncertain (The compensation dialysis method might yield somewhat more accurate results.) The bicarbonate concentration of different bloods is practically proportional to the total CO₂ content at the same CO₂ tension, and hence Van Slyke's method of determining the alkaline reserve of plasma might be used to determine the bicarbonate, provided the conversion factors from his units to titration units were known. The bicarbonate content of blood does not often exceed 0.04 N, as will be shown in a later paper.

Since the concentration of neutral salts is only about a fourth as great as in sea water, their effect in reducing the hydrolysis is slight. The bicarbonate concentration is probably but little more than that of a pure bicarbonate solution that has the same pH at the same CO_2 tension and temperature, and hence could be estimated by means of a chart constructed on the same principles as Fig. 3 but somewhat more accurate in this region.

The above views seem to differ somewhat from those of Henderson and Cohn on sea water. Henderson and Cohn found it necessary to add 0.0015 M H_3BO_3 to a liter of alkaline NaCl solution in order to make it behave like sea water in regard to pH. My experiments were at variance to this, but since CO_2 tension is one of the most difficult factors to determine exactly, other methods seemed necessary to determine the concentration of non-volatile buffers in sea water. A serviceable method was found to be the titration of CO_2 -free sea water with CO_2 -free NaOH in the hydrogen electrode. It is difficult to maintain the sea water and NaOH absolutely CO_2 -free, and the first trace of CO_2 is immediately titrated as non-volatile buffer. By titrating directly into the electrode shown in Fig. 5 (after removal of the trap at the top) the results could be closely duplicated, and are shown in Fig. 6. The titration must be done rapidly and not carried beyond pH = 10 owing to the precipitation of (carbonates if present) phosphates (borates?) and finally hydroxides of Ca and Mg. Sea water has hardly more non-volatile buffer than artificial sea water previously described (McClendon, 1916, b). The concentration of non-volatile buffer in Atlantic, Pacific, and Gulf Stream water is practically identical. On the contrary, the non-volatile buffer in the solution used by Henderson and Cohn is very much higher in concentration. Boric acid was detected in all samples of sea water, but it is evidently in less concentration than 0.0015 M. The phosphoric acid quantitatively recovered from sea water is negligible.

In attempting to confirm these findings by plotting the pH against the CO_2 tension, as was done by Henderson and Cohn, the first experiments were apparently vitiated by the presence of some air in the CO_2 . At any rate very irregular results were obtained. In an attempt to clear up the doubt aroused by these results, a large number of determinations were made on a varied

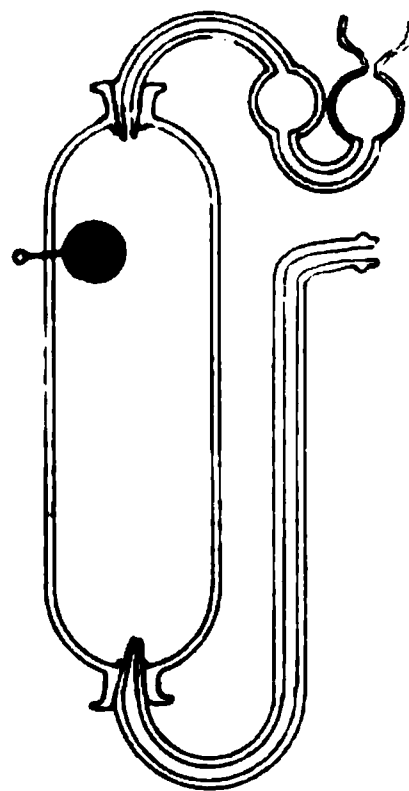


FIG. 5. Hydrogen electrode for CO₂-free titrations, for standardizing buffer mixtures, and determining the salt action on indicators (for which purpose its diameter is made the same as the colorimetric tubes). The tube admitting hydrogen at the bottom is ground to fit the openings at each end of the electrode, so that the latter may be inverted when it is desired to change the height of the palladiumized disk. In making the electrometric titration the trap at the top is discarded, and the burette tip inserted in its place. Glass wool moistened with distilled water and wrapped around the burette tip serves as a trap to keep out O₂ from the air above. The lower ground joint is ungreased because it is immersed in the KCl bath for electrolytic connection with the calomel electrode.

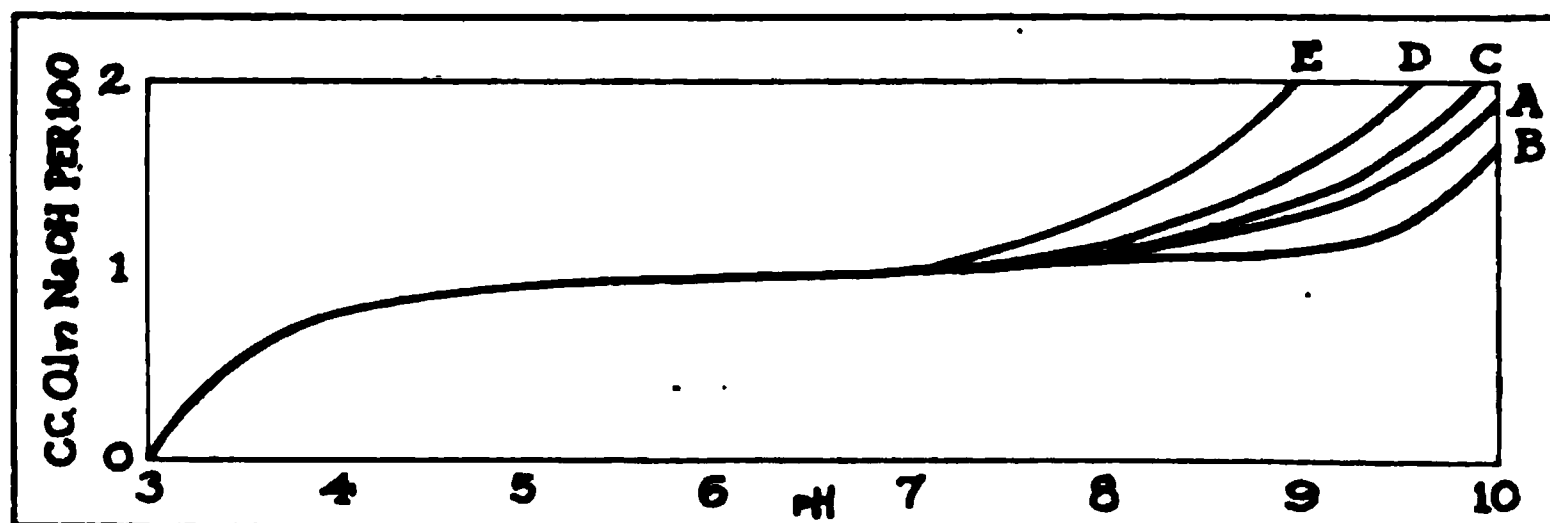


FIG. 6. Electrometric titration of CO₂-free sea water and artificial sea waters. The pH is measured on the abscissæ and the number of cc. of CO₂-free NaOH on the ordinates. The left hand ends of all the curves practically coincide, the right hand ends are marked as follows: A = sea water. B = artificial sea water of formula given by McClendon (1916, b). C = the same + 0.00017 M H₃PO₄. D = the same + 0.001 M H₃BO₃. E = the same + 0.0015 M H₃BO₃.

series of solutions of alkaline reserve = 25, given in the following list:

- A. 0.0025 N NaHCO_3 .
- B. 0.0025 " " + 0.025 N NaCl .
- C. 0.0025 " " + 0.0025 " CaCl_2 .
- D. 0.0025 " " + 0.025 " "
- E. 0.0025 " " + 0.025 " MgCl_2 .
- F. Artificial sea water, alkaline reserve = 25 (for formula see McClendon, 1916, b).
- G. Artificial sea water + 0.0015 M H_3BO_3 .
- H. " " " + 0.001 " "
- I. " " " + 0.0008 " "
- J. " " " + 0.00017 " H_3PO_4 .

Some of these experiments indicate that $\frac{1}{2}$ Ca^{++} reduces the hydrolysis of the bicarbonate more than Na^+ does, but this note is made merely as a suggestion for further research. The experiments in general indicate that small amounts of neutral salts or non-volatile buffers have little effect on the pH at constant CO_2 tension. Some deviations from this rule were ascribed to impurities in the salts. The CaCl_2 used in the last experiments was dissolved in absolute alcohol that had been redistilled over sodium, evaporated, fused in a platinum dish, dissolved in distilled water, and carefully neutralized.

Henderson and Cohn, using the indicator method of Palitzsch, record an effect of salinity on the pH of sea water at constant CO_2 tension. It is not clear whether they mean a simple change in the concentration of neutral salts or whether the alkaline reserve was also changed. If the neutral salts alone were changed, the change in pH was in the wrong direction for the effect of salts on the hydrolysis of bicarbonate, but was in the right direction for the salt effect on phthalein indicators. According to my experiments, neither the salinity nor the alkaline reserve in sea water of the tropical or temperate oceans change sufficiently to change noticeably the relation of pH to CO_2 tension, although the alkaline reserve does change sufficiently to affect the total CO_2 greatly.

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**TABLES FOR FINDING THE ALKALINE RESERVE
OF BLOOD SERUM, IN HEALTH AND IN
ACIDOSIS, FROM THE TOTAL CO₂ OR
THE ALVEOLAR CO₂ OR THE pH
AT KNOWN CO₂ TENSION**

BY

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REPRINTED FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY

VOL. XXXI, No. 3, SEPTEMBER, 1917

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School, Minneapolis.)*

(Received for publication, May 30, 1917.)

The object of the present paper is the comparison of the results obtained by means of the hydrogen electrode and with alveolar CO₂ apparatus with those obtained by means of the Van Slyke apparatus for total CO₂ in plasma. Van Slyke has given us a factor for roughly finding the alveolar CO₂ from the plasma CO₂, but this does not help us interpret the determinations with the hydrogen electrode, and we hope that the following tables may elucidate the data on acidosis and give some basis for analysis of such investigations as those of Peters.

Since the alkaline reserve has been expressed in various units in papers by the senior author it is necessary to state that in the present paper we mean the sum of the equivalents of strong bases minus the sum of the equivalents of strong acid in the serum, expressed as a fraction of a normal solution. It is the excess base or titratable alkalinity, where the precautions described below are taken to insure the correct end-point in the titration, *viz.*, the pH of distilled water at the same CO₂ tension.

It appears to be approximately true that blood serum behaves as a bicarbonate solution made isotonic by the addition of NaCl in regard to pH and CO₂ tension (McClendon, *a*, *b*). That is to say, the non-volatile buffers, phosphates and proteins, do not have an easily measurable effect on the pH at 42 mm. CO₂ tension. The concentration of diffusible phosphates is about 0.001 M in serum, and failure to detect their effect on the pH is due to

their low concentration. The concentration of protein is less in serum than in plasma, but the sodium oxalate necessary to prevent coagulation of plasma is reduced to alkali by ashing in the compensation dialysis method of determining the alkaline reserve (*i.e.*, dialyzing serum against NaHCO_3 solutions made isotonic with NaCl and finding the solution not changed by dialysis). Hence we confined our studies to serum, in order to have a uniform material for all experiments.

EXPERIMENTAL.

A modified Ringer's fluid of the following composition behaves as serum of the same alkaline reserve in regard to pH, CO_2 tension, and total CO_2 : 0.7 per cent NaCl , 0.04 per cent KCl , 0.02 per cent CaCl_2 , 0.027 per cent $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 per cent NaHCO_3 , 0.001 M H_3PO_4 . We have used this fluid in experiments on living animals with gratifying results. It should not remain open to the air, as it thus becomes too alkaline.

Our first problem was the determination of the alkaline reserve of the serum to be used in further work. We tried the compensation dialysis method of Michaelis and Rona, and found the alkaline reserve of ox serum apparently to vary from 0.03 to 0.04 N, but the osmotic pressure of the proteins and the varying permeability of the collodion membranes introduced errors, and our results may be too high. If serum is titrated with acid, using the hydrogen electrode as indicator, some means must be taken to remove the CO_2 or keep its tension constant in order to determine the correct end-point in the titration. If hydrogen is bubbled through the serum in an ordinary hydrogen electrode, some serum is carried away in the foam. In order to obviate this difficulty, we used the rotating hydrogen electrode previously used with bicarbonate solutions (McClendon, *b*). Volumetric flasks of the same size were filled with the serum and a different quantity of acid was added to each flask. The flasks were shaken and portions of the serum transferred successively from each flask to the rotating electrode, and a stream of the gas was passed through. The pH of the serum from each flask as well as the acid added was recorded, and these data were used to construct a titration curve from which the end-point in the titration could be

determined with accuracy (being the pH of distilled water at the same CO_2 tension). Owing to the time required for a single titration, we made no attempt to get the normal alkaline reserve of any species of animal by this means. The blood had been defibrinated in open dishes, and CO_2 allowed to escape. It is well known that the alkaline reserve of serum decreases when CO_2 escapes from the defibrinated blood, due to an ionic exchange with the corpuscles. The results reported apply to serum prepared as described.

Having determined the alkaline reserve of a series of sera, we added NaHCO_3 or acid to portions of serum to increase the range of alkaline reserve.

The experiments were made in a room kept at 20° by means of an electric apparatus. On a few days when the outside temperature rose above 20° the room was cooled by means of ice placed in front of an electric fan. A highly sensitive galvanometer was used with the potentiometer.

About 1.5 cc. of serum was placed in the rotating electrode, and a mixture of H_2 and CO_2 passed through it from the gas mixer. The stop-cocks were closed and the pH was determined, then the serum was used for the determination of the total CO_2 in the Van Slyke apparatus. In order to avoid some loss of CO_2 that always occurred when the serum was measured in a pipette, the serum was allowed to run quickly into the cup of the Van Slyke apparatus and a measured quantity allowed to run down into the graduated portion. The remainder of the serum was washed out of the cup with distilled water and the cup dried with filter paper. A correction was applied for the reversal in curvature of the meniscus and the volume of the hole in the stop-cock. 0.6 N HCl was introduced into the apparatus until the total fluid was 2.5 cc. This concentration of HCl has about the same absorption coefficient as serum for CO_2 (0.822 at 20°). The determination was that recommended by Van Slyke. The CO_2 pumped out was absorbed by NaOH and the CO_2 remaining in the acidified serum was calculated in each determination, then the total CO_2 was reduced to 0° and 760 mm.

The results of the experiments are given in Figs. 1 and 2. From Fig. 1 the pH may be determined from the CO_2 tension and alkaline reserve, or the CO_2 tension may be determined from the

pH and alkaline reserve, or the alkaline reserve may be determined from the pH and CO_2 tension.

The alkaline reserve may be approximately determined from the alveolar CO_2 tension alone, since the pH of blood in the arteries is remarkably constant, being about 7.33-7.5 (the variation being due largely to the method of determination). If the serum is allowed to come to equilibrium with the alveolar air at 20° the

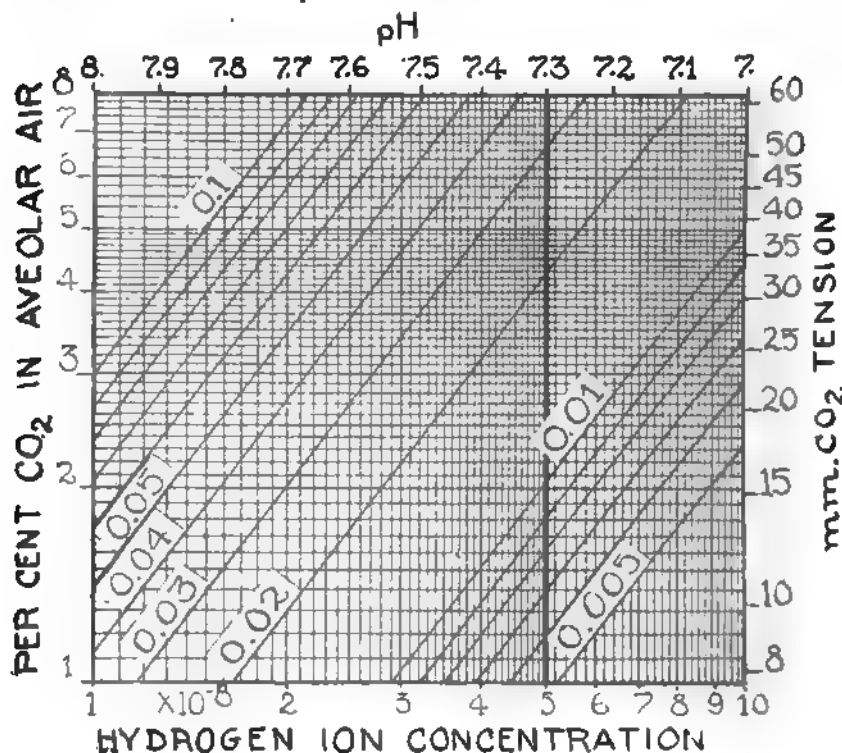


FIG. 1

pH is about 0.15 lower and in our experiments was 7.3; hence the alkaline reserve is represented by that diagonal passing through the intersection of the alveolar CO_2 abscissa with the pH 7.3 ordinate. In other words the alkaline reserve is nearly half the alveolar CO_2 tension in atmospheres, and can be determined more accurately than this from Fig. 1. In order to bring the blood or serum at 20° to the same pH as in the arteries, its CO_2 tension

must be lowered about 25 per cent. If the blood is drawn from the artery directly into a tube that may be closed so as to prevent

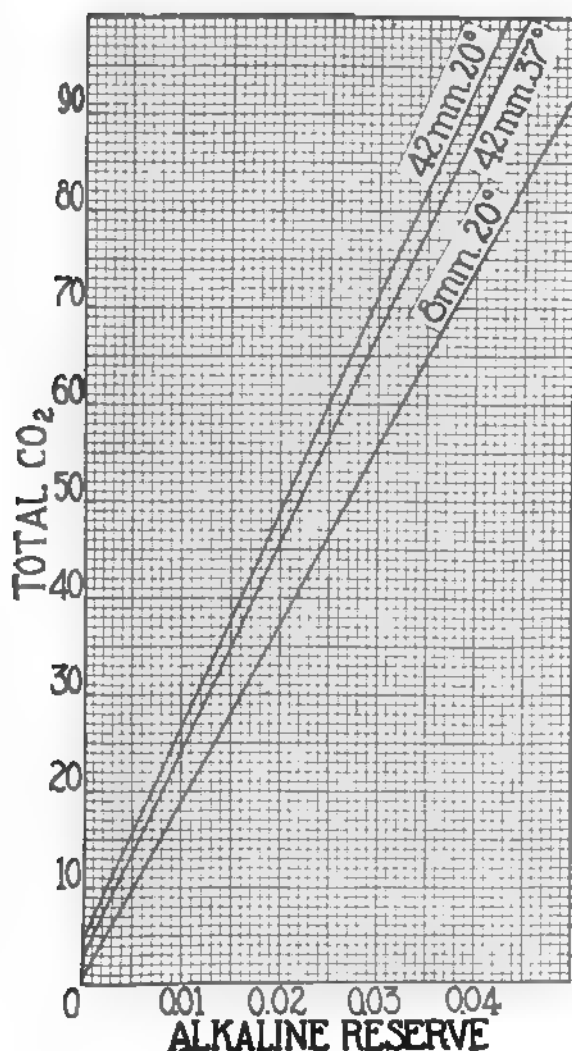


FIG. 2

gain or loss of CO₂ and cooled to 20°, the pH remains the same but the CO₂ tension falls about 25 per cent. Fig. 1 would represent the relations at 37° if the numbering of the pH scale at the

top were increased by about 0.15, or so that the heavy ordinate is numbered about pH 7.45 instead of 7.3. That is to say, the numerical relations between alkaline reserve and CO_2 tension remain the same, and it seems unnecessary to draw another figure for 37° . The rise of 0.15 pH due to rise in temperature from 20° to 30° at constant CO_2 tension is uncertain. We obtained values from 0.11 to 0.17 on serum, blood, and bicarbonate solutions. For a given difference of temperature the rise is less the higher the initial temperature.

Because war conditions have separated the authors from each other and from some of their notes, it is impossible to give the results in detail, but it seems worth while to report at present the mean of the results obtained.

From Fig. 2 the alkaline reserve may be determined from the total CO_2 at a given CO_2 tension and temperature, but a slight error in tension or temperature has little effect on the total CO_2 . (The effect of change in temperature was calculated from a large number of experiments on bicarbonate solutions.) The data may be condensed into the following empirical formulas:

$$\begin{array}{l} 20^\circ, 42 \text{ mm. } \text{CO}_2 \text{ tension, total } \text{CO}_2 = 4.5 + 2,180 \times \text{alkaline reserve.} \\ 20^\circ, 8 \text{ " " " " " " } = 0.87 + 1,820 \times \text{" " " " " " } \\ 37^\circ, 42 \text{ " " " " " " } = 2.9 + 2,155 \times \text{" " " " " " } \end{array}$$

If arterial blood is drawn into a tube in such a way that the serum may be collected without loss of CO_2 , the alkaline reserve may be calculated from the total CO_2 , by using the formula:

$$\text{pH } 7.5, \text{ total } \text{CO}_2 = 2,250 \times \text{alkaline reserve.}$$

If serum is subjected to alveolar CO_2 tension at 20° the alkaline reserve may be calculated from the formula:

$$\text{pH } 7.3, \text{ total } \text{CO}_2 = 2,333 \times \text{alkaline reserve.}$$

If the CO_2 is removed from serum until the pH rises to 8, the alkaline reserve may be calculated from the formula:

$$\text{pH } 8, \text{ total } \text{CO}_2 = 1,850 \times \text{alkaline reserve.}$$

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Since this paper was written the attention of the senior author has been called to a paper by Hasselbalch (*Biochem. Z.*, 1916, lxxviii, 112). Hasselbalch's observation that rise of temperature does not change pH if CO₂ does not escape was previously observed by McClendon and Magoon (and is also true of bicarbonate solutions). Hasselbalch's observation that rise in temperature at constant CO₂ tension increases the pH of bicarbonate solutions is also true of blood. His observation that corpuscles behave as buffers was previously published by McClendon and Magoon and is explained perhaps by the ionic exchange between corpuscles and plasma. Obviously the pH of the corpuscle interior is not measured by the hydrogen electrode, but a change in pH of corpuscle may cause a change in pH of plasma or serum.

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**THE USE OF THE VAN SLYKE CO, APPARATUS
FOR THE DETERMINATION OF TOTAL
CO₂ IN SEA WATER**

**BY
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**(FROM THE PHYSIOLOGICAL LABORATORY OF THE UNIVERSITY OF
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**REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXX, No. 2, JUNE, 1917**

THE USE OF THE VAN SLYKE CO₂ APPARATUS FOR THE DETERMINATION OF TOTAL CO₂ IN SEA WATER.

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Minneapolis.)*

(Received for publication, November 27, 1916.)

Since the apparatus for very accurate determinations of the CO₂ in aqueous fluids has usually been quite complicated and expensive, the present investigation was undertaken to determine the degree of accuracy that might be attained with Van Slyke's simplified mercury pump and gas burette, with special reference to sea water. With careful manipulation, an accuracy of 0.5 per cent was attained. In order to accomplish this, a large number of corrections had to be applied, and since these corrections vary so much for different determinations that they cannot be combined in a table such as Van Slyke has made for blood, an example of the results of a determination with the corrections applied is given below.

A solution of double normal HCl and one of half normal NaOH were prepared, to liberate and absorb the CO₂. The elementary gases have about the same solubilities in these solutions as in sea water, and the solutions were brought to equilibrium with the elementary gases at the same partial pressures as in sea water. In this way, any liberation or absorption of inert gas, due to mixing these solutions with sea water in the apparatus, was avoided.

The stock form of apparatus from Emil Greiner Company was used, but with a leveling bulb with perpendicular sides, in order to prevent an error in reading the height of the mercury. This cylindrical bulb was connected to the apparatus by means of a piece of vacuum tubing of soft rubber and of only 1 mm. bore, so as to reduce its weight and thus prevent interference

with vigorous shaking. The bulb was attached by means of a swivel joint to a screw of about 4 mm. pitch that was passed through a nut held in a burette clamp. By means of the screw, very accurate adjustment of level could be made.

The apparatus was cleaned with potassium bichromate in concentrated sulfuric acid, rinsed with distilled water, and the stop-cocks were greased with a preparation made by dissolving native rubber in boiling paraffin and adding vaseline in order to obtain the right consistency. The apparatus was filled with mercury in the usual manner, to about 2 mm. above the upper stop-cock, and evacuated once in order to remove air bubbles. It was refilled with mercury, care being taken that both capillary tubes above the upper stop-cock were filled. 10 cc. of sea water were measured in a pipette and introduced into the apparatus through the cup on top. In order to prevent error due to a drop of sea water remaining in the cup, only 1 cc. was admitted before the stop-cock was opened, and then the water was allowed to leave the cup as fast as it entered it. When the water level sank to the entrance of the capillary at the base of the cup, 1 cc. of double normal HCl was admitted, and the last drop of sea water washed into the apparatus with it. When the acid level sank to the entrance of the capillary, mercury was placed in the cup and allowed to pass into the apparatus, thus forcing in the last drop of acid. Care was taken that enough mercury was left in the two capillaries above the upper stop-cock in order to seal it. The apparatus was exhausted until the mercury reached the 50 cc. mark, the lower cock closed, and the apparatus inverted in order to see whether the upper stop-cock leaked. It was then held with the fingers far out at the extreme ends of the apparatus, in a horizontal position and shaken violently for 2 minutes or given 400 double vibrations, in order to bring the water into equilibrium with the reduced CO₂ pressure. Several times during this process the residual mercury was allowed to run into the graduated part of the apparatus in order to expel any sea water that thus escaped the agitation, but the time required for this was counted out of the shaking. The apparatus was clamped upright, the lower cock opened in the usual manner, and the water allowed to run into the trap below. By carefully adjusting the screw, the water level was made to coin-

cide exactly with the upper entrance of the hole in the lower stop-cock stopper, and held there 1 minute in order to allow the apparatus to drain. The stop-cock was reversed and the mercury allowed to rise until it reached the same level as that in the leveling bulb that was raised to meet it. The mercury was held at this level 1 minute in order to allow the water trapped between the mercury and the glass to rise. The volume of water above the mercury was accurately observed and recorded, and the bulb raised so as to compress the gas by half the volume of this water. The lower stop-cock was now closed and the apparatus held (the fingers being at the extreme ends) on its side so that the sea water trapped below would not pass the Y-joint, and so that the gas and residual water above the mercury would pass into the wide part of the apparatus. It was agitated 1 minute in order to bring the residual water into equilibrium with the gas. The apparatus was clamped upright, care being taken to see that the mercury in the bulb was at the same level as that in the gas burette, and its level noted. The lower stop-cock was then opened. If the mercury in the burette changes more than 0.5 mm. the levels must be adjusted and the shaking repeated, but this seldom happens provided there is less than 0.1 cc. of water above the mercury. The mercury levels are now adjusted with the most extreme care, by means of the screw. In doing this for the first time, it is advisable to place two specks of dust on the mercury in the leveling bulb and sight over both of them simultaneously, in order to determine the horizontal. The leveling bulb is placed just behind the burette, and the screw adjusted so that the mercury meniscus in the burette and the two specks of dust in the bulb are brought into line. The volume of the gas is now accurately determined by reading the top of the water meniscus, and recorded. 0.5 cc. of half normal NaOH is placed in the cup and run into the burette. As the gas rises through the NaOH all of the CO_2 is absorbed, and no shaking is necessary. The mercury level is adjusted as before, but allowance must be made for the liquid over it. The length of this liquid is observed and one-tenth of it added to the top of the mercury meniscus, in order to determine an imaginary point through which to sight the two specks of dust on the mercury in the bulb. The volume of the residual gas is recorded and the barometer observed and corrected.

A large number of determinations have been made, many of them with carefully predetermined CO₂ contents; one of these determinations will show how the corrections were applied.

A solution having the same salt content as ocean water was prepared in a glass-stoppered volumetric flask from neutral salts, distilled water that had been boiled and cooled in a stream of CO₂-free air, and Na₂CO₃ that had been prepared from "reagent" NaHCO₃ by heating in a platinum dish for 30 minutes, cooling in a desiccator, and weighing with standard weights. 10 cc. of this artificial sea water were calculated to contain 0.687 cc. of CO₂.

The gas burette of the apparatus was calibrated with water while in the usual position, so that no further correction had to be applied to the actual volume of the gas. The remaining corrections were taken from the tables of Landolt-Börnstein-Roth. The temperature of the room was kept within 0.1° of 20°. The absorption coefficient of the sea water for CO₂ was taken as 0.777, from the work of Bohr.¹ The barometer was compared with a standard barometer, and corrected for gravity. Since there was 2 mm. capillary depression in the gas burette, this amount was subtracted from the corrected barometer reading, before making the calculations.

The volume of sea water above the mercury was read at 0.065 cc., but 0.014 (the combined volumes of the two menisci) must be added, making 0.079. The CO₂ as gas was equal to the total gas (0.778) minus the gas after absorption (0.195) or 0.583. The partial pressure of CO₂ on the water above the mercury was $0.583 \div 0.778 = 0.7494$ (of an atmosphere). The CO₂ absorbed by this water was $0.7494 \times 0.079 \times 0.777 = 0.04601$. Therefore the CO₂ pumped out of the water was $0.04601 + 0.583 = 0.62901$. Since this gas was expanded to 39 cc. when the mercury was lowered to the 50 cc. mark, the 11 cc. of water were under a partial pressure of $0.62901 \div 39 = 0.01613$, and contained $0.01613 \times 11 \times 0.777 = 0.1378$ cc. of CO₂. Therefore the total CO₂ was $0.62901 + 0.1378 = 0.76681$. The barometer (corrected) was 742.5 mm. and the log of the correction for temperature and pressure is about $-1+0.947196$. The log of the volume is about $-1+0.885$; therefore the total CO₂ reduced to

¹ Bohr, C., *Ann. Phys. u. Chem.*, 1899, lxxviii, 500.

0° and 760 mm. is 0.6794 or 0.2 per cent too high. It should be noted that for great accuracy, the exact Cl content or salinity of the water should be determined and the absorption coefficient varied accordingly, but for most purposes it is sufficient to take the salinity of ocean water as about 35 per thousand, or Cl content as about 19 gm. per liter, in which latter case the absorption coefficient for CO₂ is 0.765 at 20°.

TABLE I.

Correction to Be Added to the Observed Volume of Water over the Mercury, on Account of the Meniscus Above and Below.

Length of tube containing 1 cc., mm.....	50	60	70	80	90	100	110	120	130	140	150
Correction, cmm..	24	17	15	13.5	12.5	11.7	11.0	10.3	9.7	9.2	8.7

TABLE II.

Absorption Coefficient of Sea Water for CO₂ when the Temperature and Chlorine per Kilo of Sea Water Are Known.

18 gm. of Cl per kilo = 18.42 gm. per liter at 20° and 20 gm. per kilo = 20.5 gm. per liter at 20°. The absorption coefficient is given in cc. absorbed from one atmosphere of CO₂ by 1 liter of sea water.

Temperature, °C....	20	21	22	23	24	25	26	27	28	29	30
Cl = 18 per mill.....	772	750	730	718	688	667	652	638	624	610	596
Cl = 19 " "	767	745	725	714	684	663	648	634	620	606	592
Cl = 20 " "	761	740	720	710	680	659	644	630	616	602	588

Where sufficient skill is acquired to work rapidly, the second shaking and reading of the volume of water above the mercury may be omitted. A rubber tube of larger bore is used so that the mercury will rise so rapidly that little CO₂ is absorbed by the moisture on the walls. The first shaking is then the only shaking necessary and it may be done more rapidly with the apparatus in the vertical position. The absorption of CO₂ is delayed in hot weather by a film of vaseline from the stop-cock. It is better to grease the stop-cocks with a mixture of chicle, soft paraffin, and as little vaseline as possible.

THE WAVE
BALTIMORE

**THE HYDROGEN ION CONCENTRATION OF THE
CONTENTS OF THE SMALL INTESTINE**

BY

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REPRINTED FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY

VOL. XXXIV, No. 1, APRIL, 1918

THE HYDROGEN ION CONCENTRATION OF THE CONTENTS OF THE SMALL INTESTINE.

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(Received for publication, February 19, 1918.)

Although the prevailing idea is that the reaction of the small intestine is alkaline (Auerbach and Pick), it was shown by the senior author that the duodenal content of infants is distinctly acid ($\text{pH} = 3.1$), and by McClendon, Shedlov, and Thomson that the content of the ileum of pups, whether fed on milk or on solid food, is slightly acid ($\text{pH} = 5.7$ to 6.34), and that the duodenum of the adult dog has a similar hydrogen ion concentration. In the present paper we describe the determinations showing that the small intestine of the dog is slightly acid throughout its entire length.

Owing to the very rapid absorption from the dog's intestine it is sometimes difficult to get fluid out of the lower parts without the administration of some laxative, and in the following experiments magnesium sulfate was sometimes given, as indicated in the table below. The dogs were fed on a mixed diet of cooked food, and the abdominal cavity was opened under ether anesthesia, and samples of the intestinal contents were drawn directly into hydrogen electrodes, described by McClendon and Magoon, through punctures in the intestinal wall. The hydrogen ion concentration was determined with the aid of a Leeds and Northrup potentiometer and galvanometer. In the following table the dogs were numbered in the order received (first column), the number of hours after feeding at which the samples were taken is indicated in the second column, and the $\text{pH}(= -\log H_+)$ in the third column. Under "Remarks" is given the part of the intestine from which the sample was taken, and magnesium sulfate is indicated in case it was administered.



THE WAVERLY PRESS
BALTIMORE, U. S. A.

A NEW HYDROGEN ELECTRODE FOR THE ELECTROMETRIC TITRATION OF THE ALKALINE RESERVE OF BLOOD PLASMA AND OTHER FROTHING FLUIDS

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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXIII, No. 1, JANUARY, 1918

A NEW HYDROGEN ELECTRODE FOR THE ELECTRO-METRIC TITRATION OF THE ALKALINE RESERVE OF BLOOD PLASMA AND OTHER FROTHING FLUIDS.

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(Received for publication, October 12, 1917.)

Van Slyke and Cullen have devised a practical method for the determination of the bicarbonate reserve of plasma, based on Van Slyke's method for determining total CO_2 . The method presented in this paper also determines the bicarbonate, but by titration of the alkali instead of by the determination of the CO_2 of the NaHCO_3 molecule.

At present the most accurate method of titrating the blood alkali against acid to a definite end-point is afforded by the gas chain, since the end-points of indicators are rendered indefinite by the proteins present. With the electrometric technique one may choose between two modes of titration, (1) adding a definite amount of acid and measuring the change in pH, or (2) as in ordinary alkalimetry, adding as much acid from a burette as is necessary to obtain a definite pH. Cullen (1917) in a recent paper has used the former method. The latter, however, has the advantage that it permits one to choose an end-point so near the pH of circulating blood that comparatively little of the acid added combines with proteins and phosphates, so that only the bicarbonate is titrated.

In this paper apparatus is described for a convenient electrometric titration by addition of acid until a desired end-point is reached. As end-point we have chosen the pH of water, which is 7.00 at 23°. During the titration the free carbonic acid is reduced to approximately zero by washing out with hydrogen gas. Under these conditions ($\text{pH} = 7.0$, H_2CO_3 concentration = 0) all the

NaHCO_3 is changed to NaCl before the end-point is permanently attained; *i.e.*, for $\text{pH} = 7$ the ratio $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ must be about $\frac{1}{10}$.

If, by washing out the CO_2 gas, the H_2CO_3 is reduced to an infinitesimal value, the NaHCO_3 is also reduced to approximately zero.

The effect of the titration therefore is to add sufficient HCl to change all the NaHCO_3 into NaCl . The phosphates of the serum have a negligible effect on the titration. They are normally only about $\frac{1}{10}$ the molecular concentration of the bicarbonate, and change of pH from 7.4 to 7.0 changes only about one-fifth of the phosphate present from NaHPO_4 to NaH_2PO_4 . The neutralizing power exerted by the serum proteins for the same pH change is calculated by Henderson to be equal to that of about 0.001 N alkali, while the bicarbonate is normally about 30 times as concentrated. Since the effect of the chief known buffers of the serum (aside from bicarbonate), *viz.*, phosphate and proteins, on the titration is calculated at only $\frac{1}{10}$ and $\frac{1}{10}$ respectively of the bicarbonate effect, the assumption appears justified that our titration measures the serum bicarbonate with a very slight error. As a matter of fact, we find that the amounts of acid required in our electrometric titration of normal human serum are equivalent to an alkali concentration of 0.030 N, which corresponds almost exactly to the average bicarbonate concentration of normal plasma (65 volume per cent $\text{CO}_2 = 0.029 \text{ N}$) found by Van Slyke and Cullen as a result of direct gasometric determination of the CO_2 portion of the bicarbonate molecule.

In titrating plasma, I have used the rotating electrode (McClendon, 1917, *b*) thus avoiding frothing and eliminating the necessity of pumping, but it was necessary to disconnect the electrode every time more acid was added. It seemed necessary, therefore, to devise an electrode into which acid could be run and through which hydrogen (or $\text{H}_2\text{-CO}_2$ mixture) could be run without disconnection from the potentiometer.

After some preliminary forms of electrode had been tried, the rotating electrode shown in Fig. 1 was found satisfactory. The electrode vessel is cylindrical ($30 \times 36 \text{ mm.}$) with an opening 10 mm. in diameter in one end and the other end cemented to a cork pulley with sealing-wax, and the whole mounted in a wooden frame so as to rotate on a horizontal axis. Through the open end

the cylinder is passed a large glass tube that fits snugly in the
 Inside the large tube, three small glass tubes and a rubber
 are passed and cemented in with sealing-wax. The rubber

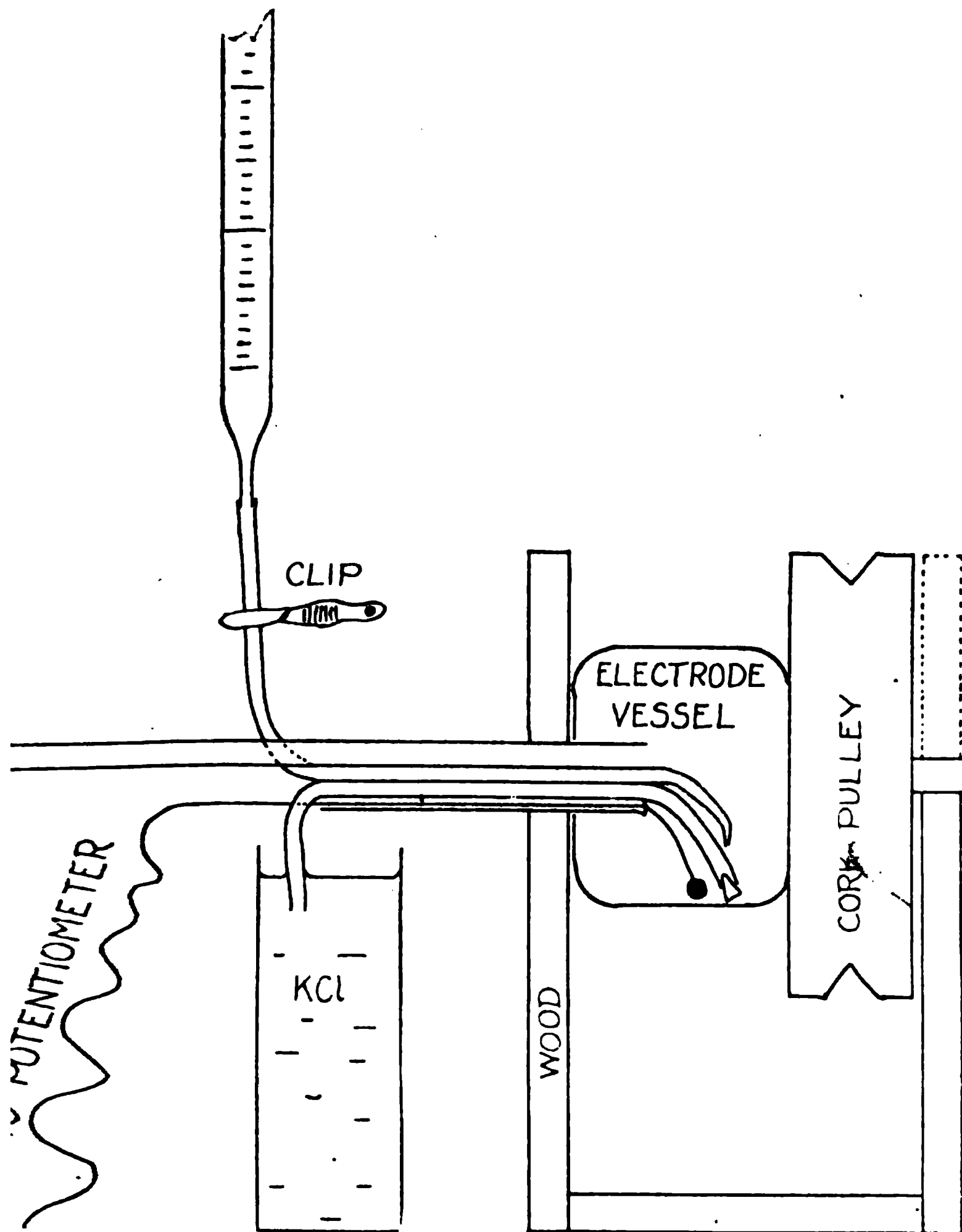


FIG. 1.

is 1 mm. bore and filled with a saturated KCl solution.
 The inner end hangs down in the cylinder and is closed with a bit
 of match that had been boiled in saturated KCl solution. The

outer end dips into a reservoir of saturated KCl solution connected with the saturated KCl-calomel electrode. The electrode proper is of gold coated with palladium black and connected to a platinum wire fused in one of the glass tubes. The wire is connected with the potentiometer. Lead glass was not used in sealing in the platinum wire owing to danger of reduction of lead thus making the platinum brittle. The 1 mm. glass tube was narrowed at one end so that the platinum wire would just go through. After insertion of the wire, a moment's heating in a tiny blast flame sealed it in the end of the glass tube. About 12 mm. of the platinum wire were allowed to protrude and a gold bead was fused on the end of it. This was accomplished by holding the end of a gold wire in a small Bunsen flame until bead of the proper size formed, and advancing the end of the platinum wire into the flame until it touched the gold bead, then withdrawing it quickly. The gold bead was hammered to a disc. The finished electrode was put in place so that the gold disc hung down in the electrode vessel. Another glass tube, 1 mm. bore was drawn to a dropping tip inside the electrode vessel and the outer end connected with a rubber tube of 1 mm. bore closed with a Langenbeck clip and connected with a 1 cc. pipette graduated in hundredths and filled with 0.1 N HCl or NaOH and used as a micro-burette. A glass tube of about 3 mm. bore served for the admission of hydrogen.

In setting up the apparatus, the frame was set in place and weighted with lead so as to remain in position. 1 cc. of plasma was run into the electrode vessel and it was put in place by inserting the axle of the cork pulley in the vertical slot in the frame made to receive it. The cork pulley was connected by means of string belt to a Tiffany motor. The micro-burette and connecting tube were filled with HCl and the rubber tube was filled with a saturated solution of KCl. The gold disc was coated with palladium black by electrolysis with a 2 volt current and a fairly strong solution of palladium chloride. When this solution became yellow it was evaporated until it appeared light brown in layer about 2 cm. thick. The electrode was sprayed a moment with distilled water and the 10 mm. glass tube containing the four smaller tubes carefully inserted through the hole in the wooden frame and the hole in the electrode vessel. The 10 mm. tube fits tight in the hole in the frame and, owing to the downward

bending of the small tubes, cannot be shoved straight in but must be started at an angle, and during the process the disc must not be allowed to dry or to touch any solid object. The operation is easier than it may seem. The 3 mm. glass tube is connected with a supply of hydrogen washed with HgCl_2 , alkaline pyrogallol, and distilled water, and a vigorous stream of the gas passed through the electrode vessel. The Tiffany motor is started and the electrode vessel rotated so as to spread the plasma on its walls, at the same time leaving enough in the bottom to cover the disc. If any portion of the disc dries or gets a clot on it, it must be cleaned and recovered with palladium black. Readings are taken with the potentiometer and acid is run in while the vessel rotates. A 455 ohm galvanometer was used and a balance with the potentiometer took but a few seconds. The palladium black was removed each day by inserting the disc for a moment in aqua regia and spraying with distilled water, and was then deposited again. Since this can be done so quickly and without disconnecting the pipette or hydrogen tube or emptying the rubber tube of a saturated solution of KCl, I have not attempted to determine the length of life of a coating of palladium black. Platinum black has lasted longer in other electrodes but is not so easily removed. The gold disc may be dispensed with, but in order to get surface it is well to use a thick platinum wire, and a thick wire is liable to cause the 1 mm. glass tube to crack. I tried sealing a thick platinum wire into the glass tube with paraffin, and found that it worked for a few determinations. It could be coated with platinum black and cleaned by pulling it out of the paraffin and holding it in a flame. In putting it back, if great care was not used to see that the paraffin seal was perfect, moisture sometimes got into the glass tube and threw out the readings. Some platonic chloride solution that had been used for conductivity electrodes and had perhaps been contaminated with heavy metals, when used to platinize the hydrogen electrode, gave erroneous readings.

The end-point chosen for the titration was the pH of pure water which is about 7.03 at 20°, 7.00 at 22–23°, and 6.95 at 25°. The titrations were made at 23°.

The time required for a titration is not prohibitive for routine work. The collection, oxalation, and centrifugation of the blood under precautions to avoid the loss of CO_2 must be done in any

method. Measuring 1 cc. of the oxalated plasma into the electrode vessel and cleaning and recoating the disc takes but a few moments. If 0.4 cc. of 0.1 N HCl is added at the start the titration back with alkali takes 4 minutes. If the titration is made with HCl the speed with which the pH rises after the addition of acid indicates the amount of acid that it is safe to drop in the next time. The following example shows the time required for titration to the nearest hundredth of a cc. The final two pH readings give a possible method for estimating to thousandths of a cc., but whether such an estimation can be duplicated has not been determined. In some cases a weaker HCl solution was used to ensure more accurate volumetric measurement. In the following table, since seconds are not recorded, in some cases, two operations are placed opposite the same minute.

Hour, p.m.	Burette reading.	pH	Hour, p.m.	Burette reading.	pH
3 49	0.1		3.54		6.96
3 50		7.50	3.55		7.00
3 50	0.2		3.56		7.02
3 51		7.30	3.56	0.31	
3 51	0.3		3.57		6.85
3 52		6.50	4.00		6.87
3 53		6.90	4.05		6.87

The titration in this case took 16 minutes and the alkaline reserve was found to be between 0.03 and 0.031 N. It is not always possible to find the end-point with only four additions of acid, but it is only after the last two burette readings that it is necessary to wait for the definitive pH to be reached. In the following titration six additions of acid were required but the time spent was the same.

Hour, a.m.	Burette reading.	pH	Hour, a.m.	Burette reading.	pH
10 15	0 1		10 21		7.05
10 16		7.4	10 21	0.32	
10 16	0 2		10 22		6.95
10 17		7.20	10 25		7.05
10 17	0 25		10 25	0 33	
10 18		7.10	10 26		6.85
10 18	0 30		10 30		6.85
10 19		6.70			

The whole of 15 minutes need not be spent in titration as it is possible so to regulate the readings that practically all of the last 10 minutes may be spent in doing other things.

In the above titrations, the pH of pure water was taken arbitrarily as the end-point. This method gives at least comparative values and probably is not far from the true end-point, since it should at least determine the bicarbonate. It is impossible to titrate the phosphates, as a pH of about 3 to 4 would be necessary to decompose them, and at this pH the proteins would bind acid. The isoelectric point of serum albumin is at $\text{pH} = 4.7$, and of serum globulin $\text{pH} = 5.4$, and these proteins should not bind acid or alkali at their isoelectric points. These proteins may bind some alkali at $\text{pH} = 7$ and therefore it might seem best to use the mean of their isoelectric points as the end-point for titration. It is necessary, however, to use about a third more acid in order to do this and it seems improbable that the proteins bind a fourth of the alkali at $\text{pH} = 7$. There may be other ampholytes in the plasma with very different isoelectric points, since the concentration of diffusible phosphates is not sufficient to account for this large acid-binding power. According to a rough calculation, the pH of distilled water under an atmosphere containing 5 per cent CO_2 should be near the isoelectric points of serum proteins. It would seem of interest, therefore, to compare the titration of plasma under hydrogen with that under hydrogen containing 5 per cent CO_2 . Owing to the fact that no tanks were at hand and my gas mixer holds only 1 liter, I was not sure that equilibrium was reached. A sample of plasma was titrated while H_2 was passing and $\text{pH} = 7.00$ was maintained after 0.33 cc. of acid had been run in. By means of a 3-way cock, $\text{H}_2 + 5$ per cent CO_2 was substituted and the change in pH noted while a liter of the mixture passed through the electrode vessel. The pH gradually fell, finally reaching 6.3. Since this is still far removed from the isoelectric point of the proteins, it seems probable that some other buffer action is present but whether the phosphates could account for this buffer action was not determined.

In the electrometric titration of many solutions the end-point is marked by a more or less distinct angle in the pH curve and it was thought advisable to plot these curves for plasma. The curves plotted by Cullen are almost straight lines and therefore

it would be necessary to remove more of the CO₂ than is accomplished by Cullen's method in order to show an angle or an asymptote. This removal of CO₂ is rapid at first but becomes slower and slower.

In the following table the number of minutes between dropping in the acid and measuring the pH is recorded.

Min.	pH	Burette reading.	Min.	pH	Burette reading.
00	8.30	0.2	00		0.3
1	6.70		1	7.20	
5	7.00		10	7.35	
10	7.13		15	7.35	
15	7.36		00		0.4
20	7.50		1	6.00	
25	7.65		5	6.05	
35	7.93		10	6.07	
40	8.02		15	6.07	

The above table merely shows that 40 minutes is not enough for the elimination of the CO₂ when an appreciable amount of the alkali is left in the plasma. The following table shows a similar experiment.

Min.	pH	Burette reading.	Min.	pH	Burette reading.
00	8.04	0.10	10	7.30	
1	7.30		15	7.31	
2	7.65		00		0.31
4	7.80		1	6.65	
5	7.85		5	6.73	
10	7.90		10	6.73	
15	7.92		00		0.35
20	7.93		1	6.15	
30	7.94		10	6.15	
00		0.20	00		0.40
1	7.00		1	5.45	
2	7.45		5	5.50	
10	7.55		00		0.50
20	7.56		1	4.75	
00		0.25	13	4.75	
1	7.10		00		0.60
3	7.20		1	4.40	
			7	4.40	

In the above table the rate of rise of pH with washing out of CO_2 was slower than in the previous experiment, probably due to less rapid flow of hydrogen. Somewhere between pH 5.4 and 4.4 the proteins coagulated, presumably at the isoelectric point. This experiment shows the acid-binding power of the proteins after their isoelectric points have been reached by the slow change in pH on dropping in more acid, the last 0.1 cc. of acid changing the pH only 0.35.

It seems to be impracticable to determine the alkaline side of the titration curve of plasma on titration with acid, and a far better way is the addition of an excess of acid at first and titration with CO_2 -free NaOH. In the following experiment, 0.4 cc. of 0.1 N HCl were added to 1 cc. of plasma and titrated with 0.1 N NaOH. The blood had been exposed to air and hence the alkaline reserve of the plasma was not normal.

NaOH	pH	NaOH	pH
cc.		cc.	
0.00	5.40	0.25	9.02
0.05	5.92	0.30	9.43
0.10	6.57	0.35	9.76
0.125	7.00	0.40	10.02
0.15	7.42	0.45	10.24
0.20	8.32	0.50	10.42

On plotting these data a logarithmic titration curve is produced which shows, however, considerable buffer effect. After the alkali is dropped in and well mixed by tilting the apparatus and by the rotation, a fall of potential of less than 2 millivolts is noted. This effect increases as the alkalinity increases and may be due to a slow process of combination of alkali with protein. No argument can be deduced from this curve to show that some other pH than 7 is a more logical end-point for titration. It may be noted that when the alkali equivalent of the acid is dropped in the pH rises to 10. Plasma should reach this pH on driving out the CO_2 with hydrogen, but it would take a long time to accomplish it.

On adding an excess of acid to drive out the CO_2 it is necessary to allow the vessel to rotate and a rapid stream of hydrogen to

flow about 5 minutes or more before beginning to titrate back with NaOH. The following table gives the minutes required, as shown by the rise in pH to an equilibrium.

Min.	pH	Min.	pH
1	4.75	5	5.40
2	5.15	6	5.40
3	5.30	7	5.40
4	5.35	8	5.40

Determinations may be made on 0.1 to 0.5 cc. of plasma using 0.01 N HCl or NaOH. If the plasma does not cover the disc, it must be diluted with 1 per cent KCl solution until it covers the disc.

No undoubted cases of acidosis have yet been studied although I have agreed to do so. Plasma of lowered alkaline reserve was obtained by allowing the CO_2 to escape from the blood before centrifugation. Two such experiments gave the alkaline reserve to equal 0.017 and 0.02 N respectively, but the alkaline reserve before exposure to air was not determined. As may be seen in the above pages, the normal seems to be about 0.03 if $\text{pH} = 7$ is taken as the end-point. I do not, however, know the average normal and merely suppose certain samples to be normal since they were taken from patients without symptoms of acidosis. Van Slyke and Cullen give the average normal chemically bound CO_2 as 65 volume per cent, which I interpret as 0.029 N (since 22.4 liters of CO_2 dissolved in 1 liter of 1 N NaOH would make a 1 N solution of NaHCO_3).

It was found that plasma kept for hours at room temperature and then placed in the refrigerator over night presented no difficulties the next day. Plasma kept 36 hours at room temperature poisoned the hydrogen electrode so that no readings could be made. No H_2S was detected in this plasma, and the substance affecting the electrode was not determined.

My thanks are due to members of the staff of the University Hospital for drawing samples of blood.

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THE WAVERLY
BALTIMORE, U

[Extracted from Year Book No. 18 of the Carnegie Institution of Washington (for 1919), pages 202, 203].

The Effect of Anesthetics on Basal Metabolism, by J. F. McClendon.

Owing to the difficulties in keeping metabolism down to the basal level in higher animals, comparative studies in metabolism upon forms in which this can be done are desirable. In choosing an animal for such investigations, the jellyfish *Cassiopea xamachana* was decided on, since the automatic activity of the nervous system may be abolished by cutting off the margin of the bell ~~and the respiration rate is independent of oxygen tension, except for very low tensions of oxygen.~~

In determining the rate of metabolism, 4 jelly-fish of large size (up to 15 cm. diameter) were deprived of manubrium and bell-margin and placed in sea-water in an air-tight jar of about a liter capacity and rotated in a thermostat at 30° for 1 hour. The oxygen used was determined by the Winkler method and the CO₂ given out was calculated from the alkaline reserve and changes in the hydrogen-ion concentration (expressed as PH). It was found that the neuro-muscular system in the bell was anesthetized with 0.5 per cent ether in sea-water, whereas the jelly-fish died at the end of 1 hour in 3 per cent ether and in less than an hour in 4 per cent ether. The respiratory quotient was found to be about 0.95, and since the CO₂ determinations were less accurate than those for oxygen, only the latter are given in the following table. The jelly-fish were tested 1 hour without anesthetic as a control.

Ether.	With anesthetic.	Control.
0.5 p. ct. . .	2.35 c.c. O ₂	2.4 c.c. O ₂
1 p. ct. . .	2.4	2.4
Do. . . .	1.8	1.8
Do. . . .	2.7	2.65
2 p. ct. . .	2.2	2.1
3 p. ct. . .	1.2	1.2
4 p. ct. . .	1.2	2.05

It may be seen from the above table that, within the limits of error of the method, the oxygen consumption was the same with or without ether up to 4 per cent ether, in which case death occurred before the end of the experiment. Whether this is general for all anesthetics could not be determined, owing to limited time and the fact that some anesthetics interfere with the Winkler method. Carbon dioxid is sometimes considered an anesthetic, and the following experiments were made by adding it to sea-water and then estimating the total CO₂ content from the alkaline reserve (0.025 N) and the PH (mean between PH at beginning and end of experiment).

From the above table it may be seen that the metabolism is progressively lowered with the addition of CO₂ to the sea-water. The question arises whether the CO₂ found as bicarbonates and carbonates is effective. In order

Control (P _H = 8.15).	+CO ₂ .
2.08 c.c. O ₂	2 c.c. O ₂ , P _H =6.6
2.02	1.85 6.3
2.6	1.6 5.8
1.7	0.4 5.5
1.7	0.7 5.7

to study this, another series of experiments was made by adding HCl to decompose these salts, but without addition of CO₂ gas to the sea-water, as follows:

Control.	+HCl.
2.07 c.c. O ₂	2.02 c.c. O ₂ , P _H =6.6
1.85	1.3 5.85

It may be seen from the above table that CO₂ liberated from the salts of sea-water lowers the metabolism. In order to test whether the hydrogen-ions derived from hydrolysis of CO₂ had the depressant effect, sea-water was deprived of CO₂ after exactly neutralizing the alkaline reserve with HCl and then acidified with a few drops of phosphoric acid. In the control the jelly-fish used 2.9 c.c. O₂, and in the CO₂-free sea-water of P_H = 5.9 they used 2.7, which difference is within the limit of error. We may therefore conclude that the P_H is not the controlling factor, but that CO₂ lowers the metabolism.

**EFFECT OF DIET ON THE ALKALINE RESERVE
OF THE BLOOD**

BY

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**REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. XXXVIII, No. 3, July, 1919**

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(Received for publication, May 29, 1919.)

The beneficial effects of fruits and vegetables in the diet have been recognized for a long time. Their virtue was at one time attributed to their alkaline ash by Henry C. Sherman and others. Blatherwick¹ has shown that plums, prunes, and cranberries leave acid residues in the body due to the fact that they contain benzoic acid which is not oxidized. It is true, however, that many fruits and vegetables decrease the acidity of the urine, but it has not been proven that the total advantage of them in the diet is due to this cause. In fact the more we learn about vitamins, the more their importance rises and at the same time the importance of ash constituents of food falls.

Our interest in this subject arose from the fact that the Division of Food and Nutrition of the Army paid considerable attention to the acid-base balance of diets and it seemed worth while to show whether or not it was of importance in maintaining the alkaline reserve of blood. By alkaline reserve we mean the bicarbonate concentration of the plasma on the basis of a normal solution of sodium bicarbonate.

The blood was drawn directly from a vein or artery into a tube in which enough dry potassium oxalate had been placed to make 1 mg. per cc. of blood. This was done by measuring the required quantity of 25 per cent potassium oxalate solution into the tube and evaporating it to dryness. In the case of rabbit's blood the quantity was doubled. The blood was drawn and centrifuged with practically no exposure to air or other gas. 1 cc. of plasma was placed in a rotating hydrogen electrode and titrated with 0.1 N HCl under a stream of hydrogen to neutrality ($\text{pH} = 7$).

¹ Blatherwick, N. R., *Arch. Int. Med.*, 1914, xiv, 409.

The titration electrode was an improvement on the one previously described.² It is shown in Fig. 1. The electrode vessel was made of a 50 cc. volumetric flask with the neck cut off and the base cemented to a cork pulley-wheel with sealing wax. It was supported on a frame so that the open end would fit over a short piece of tubing attached in the frame and the base was supported by the axle of the pulley. One end of the frame supported a rod holding a micro-burette. After the plasma was placed in the vessel and the latter adjusted in the frame, a plug was carefully introduced through the short section of tubing into the electrode vessel. This plug was made of a short piece of rubber tubing through which passed a bundle of tubes the whole plug being made coherent by means of sealing wax. The bundle of tubes consisted of (1) a glass tube admitting a constant stream of hydrogen, (2) the tip of the burette, (3) a minute rubber tube filled with a saturated solution of KCl and closed at the inner end with a bit of match stick, and (4) a glass tube containing a platinum wire. The glass was fused around the platinum wire at the inner end and the outer end of the wire ended in a loop to be connected with the potentiometer. The inner end of the platinum wire had been dipped in melted gold, and before each titration it was cleaned by heating in a flame and plated with iridium by electrolyzing a strong solution (about 50 per cent) of iridium chloride with a 2 volt current for a few seconds, using another platinum wire as anode.

Since an inexhaustible supply of pure hydrogen is necessary for the continued success of the method, all previous sources of hydrogen were discarded and the hydrogen generator shown in Fig. 2 was constructed. Fig. 2 is somewhat schematic and shows only one of the battery of two electrolytic cells. Each cell was made of a beaker into which was inverted a funnel raised from the bottom by means of a piece of glass rod. Two rings of No. 10 nickel wire were made a trifle smaller than the large opening of the funnel and placed, one inside and one outside the lip of the funnel and were continuous with two upright pieces of the same wire projecting out of the cell. A mixture of 30 gm. of KOH and 100 cc. of H₂O was poured into the cell until the nickel rings were covered.

² McClendon, J. F., *J. Biol. Chem.*, 1918, xxxiii, 19.

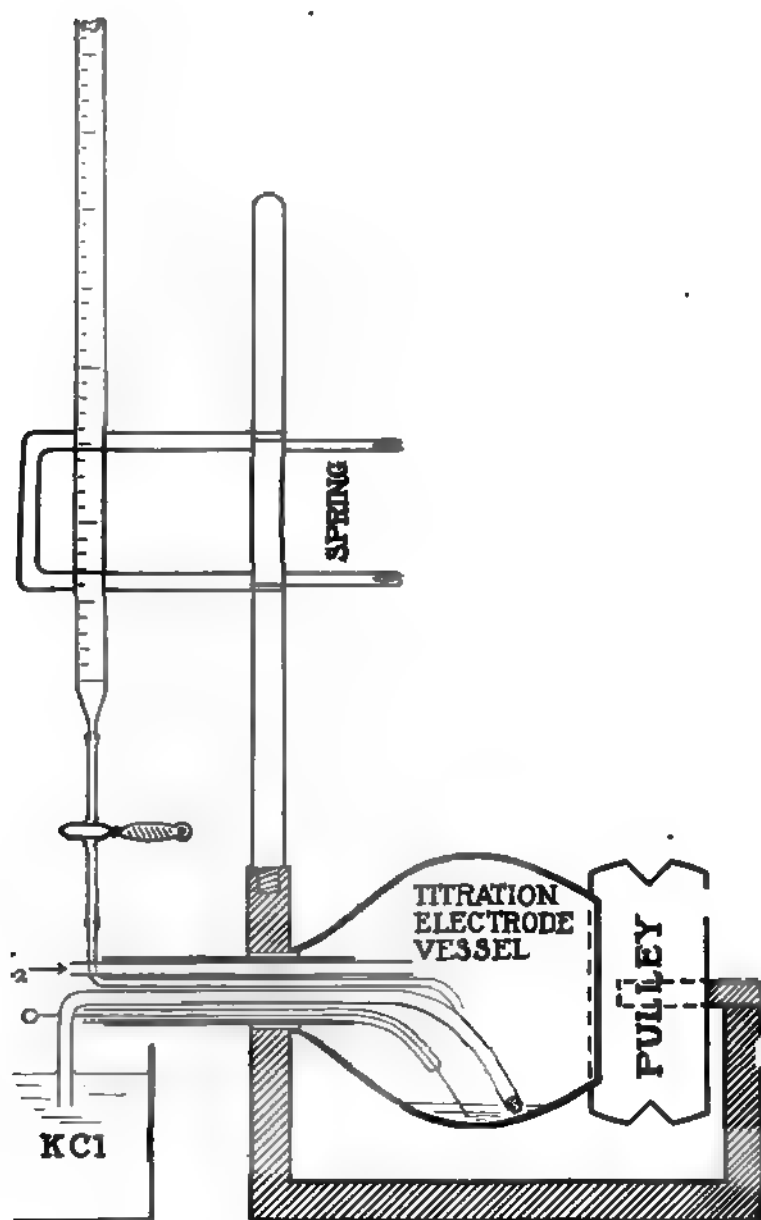


FIG. 1.

The electric current passed from one nickel ring around the lip of the funnel to the other ring with minimal heating of the electrolyte. Hydrogen was generated inside the funnel and passed up the stem and into a thick-walled rubber tube. The nickel wire

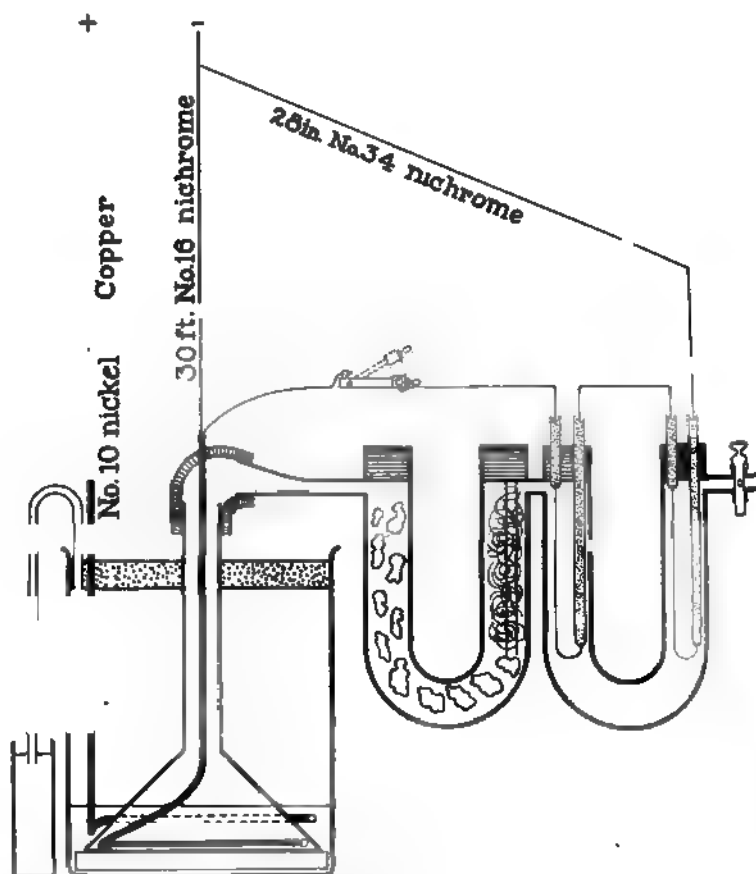


FIG. 2.

connected with the inner ring was sharpened and stuck through the rubber tube so as to connect with the source of current. The above description completes the essential parts of the generator and the additions were merely for regulating the current and pressure and removing the oxygen.

Pressure was regulated by retarding the escape of oxygen was done by closing the beaker with a sheet of metal with a thick layer of sealing wax. This cover was pierced openings, one for the funnel stem, one for the outer re, and one for a tube leading the oxygen to a trap filled er to regulate the pressure.

Hydrogen passed from the cell to a U-tube filled with CaCl_2 on and then to another U-tube, each arm of which contained-hot platinum wire 0.03 mm. in diameter to burn up an. If the gas is dried before reaching the hot wire, the med by combustion will not condense in the U-tube even , is immersed in cold water to keep it cool (as we do it). is finally passed through a wash bottle filled with 1 per H_2O to moisten it, and a spiral copper tube to bring it to erature of the room and then into the electrode.

Force of current was 110 volt direct current and the two s connected in series. The apparatus was connected to current by means of 30 feet of No. 16 nichrome wire free in the air. Part of the current which passed the cells shunted off and used to heat the platinum wires. The s of the 6 inches of platinum wire was not enough so it was by means of 28 inches of No. 34 nichrome wire. When nt was turned off from the cells the platinum wires could ated. On first starting the apparatus with air in the U-explosion would occur that would break the fine platinum in order to avoid this, a switch was placed in the shunt so air could be washed out with hydrogen before the plati-s were heated. 20 amperes of current passed through and about 5 cc. of H_2 per second were produced.

Handling of the fine platinum wire requires some care. Two s were passed through each rubber stopper of the U-tube s of larger platinum wire fused in their lower ends. A l bead was fused on the end of each wire by touching it in with a gold wire. The 0.03 mm. platinum wire was laid e gold bead and caused to adhere by reheating it for an he process repeated for the other gold bead, and then the the fine platinum wire was cut off. The glass tubes were h mercury and copper wires carrying the current dipped

In making the titrations, if enough plasma was available for triplicate determinations, the first titration was done roughly and usually overran the point of neutrality. The amount overrun was calculated from the titration curve, Fig. 3, and a second titration made by dropping in all the acid at once. Then a third titration duplicated the second. Although the titration curve in Fig. 3 is from one plasma only and may differ somewhat from other plasmas, it is very useful when applied in this way.

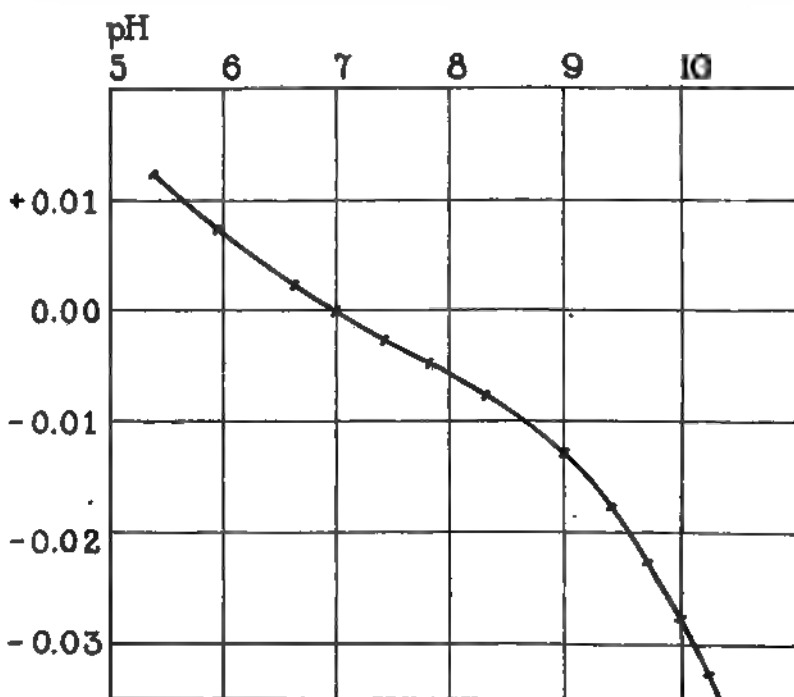


FIG. 3.

Data on the acidity or alkalinity of the ash of foods were taken from Sherman.³ Protein foods leave an acid ash due to oxidation of sulfur and phosphorus and hence meat, eggs, and all foods made of cereals are called "acid-forming." All vegetable foods except cereals contain salts of organic acids and mineral bases that yield alkaline ashes in excess of the acids from the proteins of the same

³ Sherman, H. C., Food products, New York, 1915.

foods. Butter and sugar are practically neutral and milk slightly "base-forming."

The following determinations were made on a man of 75 kilos (L. M.). L. M. was placed on an acid-forming diet for 3 days, then a mixed diet for 3 days, and then a base-forming diet for 3 days. Table I gives the results of the determinations. The other data besides the alkaline reserve are included as they might be thought to indicate something in regard to the food intake and metabolism, but except for the total acidity of the urine they were not planned for this paper. The collection of urine was commenced about 6 hours after the first meal and when blood was drawn on a certain 24 hour period, it was drawn at the end of the period. The alkaline reserve was 0.0335 before beginning the experiment.

TABLE I.

Date.	24 hour urine.			Blood sample.	
	Volume.	Titrateable acidity.	Total N.	Alkaline reserve.	Amino N per 100 cc.
1918	cc.	cc. 0.1 N	gm.	N	mg.
May 8	1,112	440	15.4		Acid-forming diet.
" 9	1,200	407	14.9		
" 10	990	417	12.3	0.0335	4.3
" 15	1,125	403	7.3	0.0355	0.7
" 16	1,360	241	8.8		Base-forming diet.
" 17	1,375	408	9.7	0.0335	1.6

It may be seen from Table I that the alkaline reserve was not changed by the acid-forming diet from the normal value for this individual (0.0335 N). After 24 hours of base-forming diet the alkaline reserve increased possibly a little more than the limits of error of the method and then went back to normal at the end of 3 days on the base-forming diet. This may have been due to ammonia formation.

Hasselbalch⁴ made some determinations on the CO₂ tension of the alveolar air on acid-forming and base-forming diets and found small differences which if they exceed the limits of error indicate

⁴ Hasselbalch, K. A., *Biochem. Z.*, 1912, xlv, 403.

that the alkaline reserve may be 0.003 N greater on a base-forming than on an acid-forming diet or during fasting. Similar determinations by Blatherwick⁵ show differences of 0.002 N. The method of reduction of CO₂ tensions to alkaline reserve values has already been described.⁶

Van Slyke, Cullen, and Stillman⁷ found that the alkaline reserve of the blood rises during gastric digestion, due to the secretion of HCl from the blood to the stomach. We drew blood at the same stage of gastric digestion (about half hour after luncheon) in each case in the hope of avoiding error due to complication from this source.

In order to use a more easily controlled diet, experiments were made on dogs. Blood was taken from the carotid arteries of four dogs and titrated. The results are given in Table II.

The fifth dog was placed in a cage March 4 and fed on 350 gm. of raw lean beef per day for 2 months and blood drawn from an ear vein at intervals and from the carotid at the end of the experiment. The blood drawn on normal diet and some specimens taken later were discarded due to clotting or getting air in the tube, but samples were correctly drawn on March 13 and May 4 and titrated 0.029 and 0.03 respectively. Since this is the average for the four normal dogs within the errors of the different methods of taking blood, we conclude that it is impossible to lower the bicarbonate concentration or alkaline reserve of dog's blood by a highly acid-forming diet for 2 months.

It was thought that rabbits might be more susceptible to changes in diet since Scott⁸ had shown that the alveolar CO₂ of rabbits varied from 4.57 to 6.3 per cent of an atmosphere. Hasselbalch⁹ gives the pH of rabbit's blood as 7.33 or very nearly what he got for man with his early technique, and, assuming it is the same as man, the above alveolar CO₂ values would place the alkaline

⁵ Blatherwick, N. R., *Arch. Int. Med.*, 1914, xiv, 445.

⁶ McClendon, J. F., Shedlov, A., and Thomson, W., *J. Biol. Chem.*, 1917, xxxi, 519.

⁷ Van Slyke, D. D., Cullen, G. E., and Stillman, E., *Proc. Soc. Exp. Biol. and Med.*, 1915, xii, 184.

⁸ Scott, F. H., *J. Physiol.*, 1908, xxxvii, 316.

⁹ Hasselbalch, K. A., and Lundsgaard, C., *Skand. Arch. Physiol.*, 1912, xxvii, 31.

reserve at 0.024 to 0.032 N. Kuriyama¹⁰ found that food changed the alkaline reserve of rabbit's blood. His figures in our units would be 0.026 N on base-forming diet and 0.021 on acid-forming diet. His results are complicated, however, by the fact that he used oats for the latter diet and oats contain no antiscorbutic substance and scurvy has been considered to be associated with acidosis. We wished to avoid this by adding fresh sprouted barley to the oat diet, as sprouted barley cures scurvy in guinea pigs. The rabbits were bled from the carotid artery under ether. The results are shown in Table III.

TABLE II.

Dog No.	Alkaline reserve.
	N
1	0.03
2	0.025
3	0.03
4	0.03

TABLE III.

Rabbit No.	Diet.	Alkaline reserve.
		N
1	Oats and sprouting barley.....	0.0145
2	" " " "	0.0175
3	" " " "	0.007
4	Fasted (water).....	0.0155
5	Carrots and hay.....	0.023
6	" (insufficient number).....	0.013
7	" and cabbage.....	0.021

These rabbits were kept on the diet 1 week except Nos. 1 and 5 which received the diet 9 days and No. 4 which received water but no food for 6 days. The results are not harmonious and the variation may be due to the different amounts of antiscorbutic substances the rabbits received in the period previous to the experiment as well as during the experiment and to partial starvation; *i.e.*, insufficient quantity of food at certain intervals. Thus

¹⁰ Kuriyama, S., *J. Biol. Chem.*, 1918, xxxiii, 215.

No. 4 which fasted showed as low an alkaline reserve as those on acid-forming diet and this is in harmony with the view that fasting induces acidosis or that the rabbit was living on its muscle tissue (as well as fat). It is evident, however, that the alkaline reserve is influenced by diet.

CONCLUSIONS.

The alkaline reserve of man and dog is remarkably resistant to influence of diet, whereas the rabbit is susceptible to the effects of diet and fasting. There is no foundation for the view that the alkaline reserve of man is endangered by acid-forming diets but such diets as usually eaten are deficient in antiscorbutic substances.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

**FACTORS INFLUENCING THE HYDROGEN ION
CONCENTRATION OF THE ILEUM**

BY

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**REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, No. 3, JULY, 1919**

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(Received for publication, May 29, 1919.)

The senior author determined the pH of duodenal contents from two adults (human) collected by Dr. Schneider in April, 1915, and found one to be 7.61 and the other to be 1.5, or an average of 4.55, whereas the average of fifteen samples from infants was 4.9. Long and Fenger¹ obtained an average of pH = 5.72 for adult human duodenal contents (thirteen samples). McClendon, Shedlov, and Thomson² obtained an average of pH = 6.2 for the contents of the ileum of seven pups and McClendon, Shedlov, and Karpman³ obtained an average of pH = 6.03 in eight determinations of the ileum of four adult dogs. The discrepancy between the results on human and dog intestine may be due to the fact, as shown below, that the intestine may become less acid on the way down and the human samples could be taken from the duodenum only.

Torrey⁴ has shown that lactose and dextrin when added to a basal diet for dogs cause replacement of *Bacillus coli* by *Bacillus acidophilus* and, although the above results may not show a difference in the pH of the lactose-eating puppy and adult dog, the human data indicate such a difference and we wished to test this question more thoroughly. Adult cats were fed as much lactose in milk as they would consume. The average of five determinations on the pH of the ileum was 6.01 as compared with 6.38 for six

¹Long, J. H., and Fenger, F., *J. Am. Chem. Soc.*, 1917, **xxxix**, 1278

²McClendon, J. F., Shedlov, A., and Thomson, W., *J. Biol. Chem.*, 1917, **xxxi**, 269.

³McClendon, J. F., Shedlov, A., and Karpman, B., *J. Biol. Chem.*, 1918, **xxxiv**, 1.

⁴Torrey, J. C., *J. Med. Research*, 1919, **xxxix**, 415.

determinations on cats fed waste food from the table. One determination on a cat to which 200 gm. of cane sugar were administered by means of the stomach tube gave $\text{pH} = 6$. A dog was fed 3 pounds of milk and 1 pound of cane sugar the first day, the same the second day, and 2 pounds of milk and 2 pounds of sugar the third day, at the end of which time the pH of the jejunum was 6, of the middle ileum 6, and of the lower or distal ileum 6.7.

In rabbits it was necessary to distinguish between different regions of the intestine, as shown in Table I which gives averages on different feeds.

TABLE I.
pH of Ileum of Rabbits.

Food.	Proximal.	Middle.	Distal.
Lactose and oats.....	6.5	6.6	6.8
Carrots	6.51	6.65	6.76
" and oats.....	6.91	7.32	7.66
Oats	7.12	7.34	7.51

The results may possibly show that the more soluble carbohydrate there is in the food, the more acid the intestine may become, yet the data do not warrant any positive statements on the subject. In fact, we began to suspect that the length of the ileum might be a factor, since in long ileums the acidity decreased on the way down.

If the ratio of the length of the ileum to that of the body is taken as an index of the relative length of the former we obtained an index of 8.6 for the cat and 13 for the rabbit, using the same methods of measurement. It is then necessary to test whether the relatively shorter intestine is more acid in all cases as it is in the above comparisons of carnivora and herbivora. In order to compare the same species we used suckling and adult rabbits. The index of the former was 10 and the latter 13. (In man the reverse change occurs, the index for new-born infants being 7.9 and for adults 3.8.) So the young ones would be expected to have a more acid ileum, as was actually found, the average values being 6.4 pH for the proximal and 6.61 for the distal portions in four young rabbits. Since this was even more acid than it was possible to make the ileum of adults by diet, we infer that the milk diet of

the young is not the only factor but that the shorter intestine is associated with more acid contents.

Since we did not find appreciable amounts of ammonia in alkaline intestinal contents, and the original pH of all the food tested was acid, we concluded that the variations in pH must be due to some other factor, such as the absorption of CO₂ as the food moved down the intestine. Long and Fenger¹ determined the pH and CO₂ tension of the intestinal contents of hogs, their average figures being as follows:

Part of ileum.	pH	CO ₂ tension in per cent of an atmosphere.
Proximal.....	6.7	23.6
Middle.....	6.96	13.0
Distal.....	7.1	14.0

It therefore appears that the intestinal contents become alkaline because the CO₂ is absorbed faster than it is produced. As the gastric juice mingles with pancreatic secretion in the duodenum, a

TABLE II.
pH of Rabbit's Ileum.

No.	Age.	Proxi- mal.	Middle.	Distal.	Diet.
1	4 weeks.	6.13		6.32	Nursing.
2	4 "	6.32		6.54	"
3	8 "	6.45		6.68	" and eating oats and carrots.
4	8 "	6.73		6.90	" " " " " "
5	Adult.	6.91	7.40	7.80	Oats and occasionally carrots.
6	"	6.84	7.31	7.72	" " " "
7	"	6.8	7.28	7.62	" " " "
8	"	6.93	7.48	7.89	" " " "
9	"	6.70	6.90	7.10	" " " "
10	"	7.40	7.71	8.10	" " " "
11	"	6.81	7.20	7.39	" " " "
12	"	7.50	7.70	7.92	"
13	"	7.00	7.18	7.25	"
14	"	6.97	7.14	7.37	"
15	"	6.81	6.90	7.00	"
16	"	6.62	6.89	7.20	Carrots.
17	"	6.09	6.28	6.34	"
18	"	6.65	6.78	6.94	Water only.

large amount of CO_2 is produced more of which is absorbed the longer the intestine. Bacterial action, however, would partially restore the CO_2 absorbed. (The mineral content of the food does not seem to be a factor in determining the pH since the acidity of the intestinal contents is greater on a diet of carrots that yield a basic ash than on a diet of oats that yield an acid ash.) That the contents of the ileum become less acid on the way down is conclusively shown by Table II.

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THE HYDROGEN ION CONCENTRATION OF FOODS

BY
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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, No. 3, JULY, 1919

THE HYDROGEN ION CONCENTRATION OF FOODS.

By J. F. McCLENDON AND PAUL F. SHARP.

(From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.)

(Received for publication, May 29, 1919.)

Owing to the fact that the vitamins of foods are more perishable in alkaline than in acid media, it was thought worth while to increase our knowledge of the hydrogen ion concentration of foods. Such determinations may also be useful in connection with a study of the hydrogen ion concentration of the contents of the intestinal tract.

Foà¹ states that the pH of ripe grape juice is 4.52, of the juice of a nearly ripe pear is 4.24, and of the milk of *Ficus elastica* is 5.7. Clark and Lubs² give a number of determinations as follows:

Substance.	Raw.	Autoclaved.
	pH	pH
Whey.....	1.64-2.56	
Vinegar.....	2.36-3.21	
Silage juice.....	3.70-3.91	
Apple “.....	3.76-5.65	3.8
Prune “.....	4.12-9.44	4.3
Beer wort.....	4.91-8.55	
Carrot juice.....	5.21-9.27	5.2
Cucumber “.....	5.08	5.1
Apple “.....	5.02	
String bean juice.....	5.23-8.63	5.2
Banana juice.....	4.62	4.6
Potato “.....	6.06-9.44	6.1
Sweet potato juice.....	5.80-8.73	
Maple syrup.....	6.75-6.8	
Beet juice.....	6.07-8.75	6.1

¹ Foà, C., *Arch. Fisiol.*, 1906, iii, 390.
² Clark, W. M., and Lubs, H. A., *J. Bacteriol.*, 1917, ii, 1.

These determinations were made with the hydrogen electrode but the juices were used to standardize colorimetric methods and were apparently kept for long periods and became more alkaline, and the more acid reaction probably represents the fresh juice. They quote the following values from the literature:

	pH
Muscle juice.....	6.8
Pancreas extract.....	5.6
Milk	6.6-7.6
Flour extract	6.0-6.5
Beer.....	3.9-4.7
Wine.....	2.8-3.8
Lime juice.....	1.7
Lemon ".....	2.2
Cherry ".....	2.5
Grapefruit ".....	3.0-3.3
Orange ".....	3.1-4.1
Rhubarb ".....	3.1
Strawberry ".....	3.4
Pineapple ".....	3.4-4.1
Tomato ".....	4.2
Plant cell sap.....	5.3-5.8

Our own determinations were begun as an attempt to find the cause of the deterioration of the antiscorbutic substance between the time of its formation in the sprouting of barley and through the process of making malt extracts in various ways. We found that the acidity of press juice or extracts of steeped barley or sprouting barley or any of the products investigated was as acid as pH = 5.5 and was not made less acid by exposure to air, filtration through fullers' earth, boiling, or evaporation at low pressure while a stream of air passed through it. This acidity is somewhat lower than orange juice and much lower than lemon juice. It might therefore be advantageous to add acid to preserve the antiscorbutic substance. It would require too much space to give all the determinations with the many variations in mode of treatment of the product, but the following example illustrates the character of the work. Barley of 95 per cent germination capacity was malted by the drum method until the acrospire was as long as the grain, the green malt was crushed between steel rollers, and a little water was added to make a pasty mass and pressed in a Buchner press without the addition of any other substance. The pH of the press juice was 5.4 and of the same after boiling 5.45.

We extended our observations to foods in general and made a long series of determinations with the result that they were all on the acid side of neutrality no matter what the condition of freshness, mode of storage, stage in the preparation (cooking), or dilution with water (extract, soup, or pot-liquor). We had supposed that meats, at least, might turn alkaline on cooking, due to the loss of CO_2 in the bicarbonates contained in them, but apparently enough lactic acid is developed in meat to prevent this change. For example, a piece of lean rabbit was boiled until the muscle fibers began to loosen and the press juice pH was 6.25 whereas the pH of the pot-liquor or water in which the meat was boiled was 5.89. We did not succeed in getting enough press juice of raw meat without the addition of water, as the clotting of the muscle proteins causes the water to be held rather firmly, but the following example may illustrate the work we did do. A rabbit was killed and the leg muscles placed in a mixture of ice and salt until frozen. The frozen muscles were quickly sliced and placed between steel plates and suddenly subjected to a pressure of 5,000 pounds per square inch to press out the muscle plasma. This muscle plasma was mixed with a little water and placed in a canvas bag in a Buchner press, and the press juice run into the electrode, showing pH = 6.

We made comparisons of the pH of vegetables pressed fresh, or first cooked and then pressed and found slight differences in the pH. In order to determine whether this was due to the volatility of some acid or base we pressed the juice from the raw food and determined the pH, then boiled the juice and then determined the pH. The differences obtained were very slight and might be due to changes in acid-binding power of proteins on coagulation.

Table I shows the pH of fruits and vegetables in our last series of determinations. The juice was pressed out of the raw food and the pH determined, then the juice was run into a silica dish, an

TABLE I.

Substance.	Raw.	After boiling.
	<i>pH</i>	<i>pH</i>
Young carrot juice	5.85	5.80
Potato juice	5.57	
Cabbage "	5.90	5.78
Orange "	3.55	3.55
Lemon "	2.32	2.30

equal volume of distilled water added, then boiled, and the original volume restored by the addition of distilled water or further boiling, as required.

In general it may be said that the juice became slightly more acid on boiling. On the contrary, if the food was boiled before pressing it might be slightly less acid, thus raw potato gave $\text{pH} = 5.57$ and boiled potato 5.79, raw cabbage 5.90 and boiled cabbage 6.50.

As the technique of hydrogen ion determinations of such material has been admirably described by Clark³ it is not necessary to go into details, but we do not believe all the precautions taken by Clark are necessary. We found it necessary to immerse the platinized part of the electrode completely but not to avoid foam, and after thorough shaking the potential was maintained constant for a long period after the shaking ceased. If the solution to be tested is sufficiently viscous to support a layer of the solution on the platinized electrode (as in the case of blood plasma), it is not necessary to take the reading with the electrode totally immersed. But in the case of most plant juices the film drains off the electrode and at the same time the potential gradually changes and continues to do so for hours and it is therefore impossible to decide the correct reading. We found that palladium-coated electrodes deteriorated so rapidly that they were discarded and platinized gold electrodes used exclusively. From other work, however, we believe iridium would be superior to platinum.

Certain unavoidable difficulties enter into the determination of raw juices that prevent absolute uniformity of results. The juices contain enzymes and, if not absolutely sterile, a variable quantity of adventitious enzymes is developed. This enzyme action may be readily observed in plant juices, due to the presence of oxidases that act on chromogens such as tyrosine. We did not make a complete study of changes in acidity due to autolysis or bacterial action, but observed isolated instances of such changes. For example, freshly pressed carrot juice gave $\text{pH} = 5.85$ whereas a portion of the same juice exposed to air 20 minutes before running into the electrode gave $\text{pH} = 5.73$. All our observations showed increase in acidity on standing, but we interpret the tables of Clark and Lubs as indicating that the juices might become alkaline, possibly due to alkaline fermentation such as occurs in urine.

³ Clark, W. M., *J. Biol. Chem.*, 1915, xxiii, 475.

THE WAVERL
BALTIMORE.

NOTE ON THE ULTRAMICROSCOPY OF EGG ALBUMIN

BY

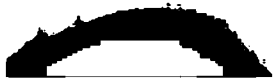
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Vol. XXXVIII, No. 3, JULY, 1919



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NOTE ON THE ULTRAMICROSCOPY OF EGG ALBUMIN.

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(Received for publication, May 29, 1919.)

The more that is known of the physical chemistry of proteins, the less they appear to resemble the suspension colloids. Sørensen¹ has studied egg albumin from the standpoint of an electrolyte, and considers the molecular weight to be about 3,400, and the pH of the lowest osmotic pressure 4 to 4.4, of the lowest solubility 4.58 (in presence of ammonium sulfate), and of the isoelectric point 4.8. He has shown that it can be purified by recrystallization and that the crystals probably consist of 2 albumin molecules, 3 molecules of sulfuric acid, and 830 water molecules.

If proteins exist in true solution we would expect ultramicroscopic particles to be absent or very numerous, depending on whether the individual molecules could be seen or not. We recrystallized egg albumin three times by Sørensen's method and made a saturated solution of the third crystals in distilled water. The pH of this solution was about 4.2 and the ultramicroscope showed only an occasional submicron. On titrating it to pH=4.8 in a hydrogen electrode by means of 0.1 N NaOH and then re-examining it with the ultramicroscope we found a slight increase in the number of submicrons, but this may be due to the fact (also observed by Sørensen) that a precipitate forms very slowly in such solutions on long standing, and we suppose the submicrons may be the first indication of a change resulting in precipitation or denaturation of the albumin. We believe that in the absence of denatured albumin there would be no submicrons present. The presence of the Tyndall effect can hardly be taken as proving the presence of colloid particles, since it has been observed in solutions of sugars that are not colloids in the usual sense of the term. It seems unfortunate to us that clear solutions of proteins should be classed with suspensoids under the term "colloids."

¹Sørensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 1917, xii.

THE WAVERL
BALTIMORE.

A CHART FOR RAPID CALCULATION OF CALORIFIC VALUES OF DIET

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The accompanying chart furnishes the calorific value per unit weight and performs simultaneously the process of multiplication and division, thus giving an immediate answer to the question of the calorific value of any diet, bill of fare or store of food. It is one thing for the physician to recommend a more adequate diet for a patient suffering from pellagra, tuberculosis or other disease, but quite another thing for the patient to procure such a diet. With the increase in cost of food, economic rationing becomes more and more desirable. The calculation of the calorific value of food is seldom done as an economic measure, since the cost of such calculation often exceeds the savings that may arise therefrom. The object of the chart is to reduce the labor of calculation to a vanishing quantity. The time required to use the chart decreases with practice, but is so small as to be negligible even the first time the chart is used. The first time I used the chart, five minutes were required to calculate the calorific value of a diet containing seventeen articles of food, and the error was only 5 per cent. The error due to variation of the foodstuff may be 5 per cent. or more, and hence the use of the chart in calculation is open to no more criticism than the use of Atwater and Bryant's data for the composition of food.

Since food is usually purchased by the pound or in containers whose capacity in pounds is known, the use of the pound as the unit of weight is most convenient. Besides the chart, a measuring tape is necessary. A millimeter tape is most convenient if it is remembered that each millimeter represents 100 calories, or each centimeter, 1,000 calories. The "calories per pound" scale at the top of the chart represents a millimeter tape with the calory units marked on it, and it may be cut out or copied for use, but it is not long enough and should be extended. A fine thread may be used in conjunction with the "calories per pound" scale, as in the ordinary practice of measuring with a string and transferring to a rule.

A list of the foods and their weights in pounds is prepared. Beginning with the first food on the list, the scale opposite its name on the chart is found and the number corresponding to its weight is noted on the scale and the distance from this number to the left hand end of the scale is measured with the tape. The part of the tape used in this measurement is marked off by covering it with the left thumb, the next succeeding portion of the tape is used to measure the second article of food, and at the end of the process, the calorific value of the whole list of foods is read off directly from the tape. For instance, let us suppose that we wish to calculate the total calorific value of 1 pound of bread and 10 pounds of apple butter. Using the millimeter tape measure (which is identical with the "calories per pound" scale at the top of the chart) we measure off one unit of the bread scale and find it to be 13 mm. Then, taking the same measure and commencing at the 13 mm. point we measure 10 units on the apple butter scale. We shall find that this will take us up to about 84 mm. Therefore, the total calorific value of the 1 pound of bread and 10 pounds of apple butter are 8,400 calories. There may be an error in calculation of 20 calories, which is only 0.5 per cent. Of course, in rapid work, ten times as great an error may occur.

Some further examples are as follows: Suppose a meal is made of 1 pound of beef and 1 pound of potatoes. We measure the beef as about 10 mm. and the potatoes as about 3 mm., or a total of 13 mm. or 1,300 calories (whereas the correct calculation is 1,307 calories by multiplication and addition). Besides this slight error in calculation there is a much greater error in using an average figure for beef. If we were calculating the beef used in an army mess, the data would be nearly correct; but when the beef is cut up there is considerable variation in the different cuts, as illustrated by the fact that Atwater and Bryant devote ten pages of tables to data on fresh beef. Since the different cuts vary from 125 calories per pound for very lean neck to 2,440 calories per pound for very fat flank, it might seem useless to use the chart for beef at all. In case of the higher calorific values, most of the calories are due to large masses of fat that would not be eaten as such, and the lower calorific values are due to the presence of much bone that makes the cuts fit only for soup. In the ordinary practice of eating steaks and chops, the average figure for beef would not be very far wrong. If we have a different scale for every cut of beef, the chart would be too large for ordinary use.

As another example, suppose we make a pudding of a dozen eggs, 5 pints of milk and half a pound of sugar. A dozen eggs weight approximately $1\frac{1}{2}$ pounds, and 5 pints of milk 5 pounds. We find that eggs have the same calorific value as canned baked beans, and milk the same as bananas, to remember which will aid in finding the scale. We mea-

Apple butter; chicken;
Apples; pears; apricot
Asparagus; canned str
Bacon
Bananas; milk; potato
Beef; lamb; turkey .
Beef sausage; prunes
Bread; dried apples, a
Butter; oleo . . .
Cabbage, canned pumpk
Canned baked beans; e
Canned, corned, or dri
Canned corn; halibut s
Canned peas
Cheese (American) .
Chocolate
Cocoa
Currants; raisins .
Codfish
Dried beans; flour; ma
Grapes
Grapefruit; oranges; f
Lard; oil; cooking fi
Sugar; oatmeal; hard
Pork; cured ham . .
Pork sausage . . .



sure off 1.5 units on the egg scale and find it to be 9 mm., and 5 units on the milk scale and find it to be 15 mm., or a total of 24 mm., and half a unit on the sugar scale and find it to be 9 mm., making a grand total of 33 mm., or 3,300 calories. The correct calculation gives 3,317 calories; hence our error is about 0.5 per cent.

If the food has been weighed in ounces, the same chart is used, but a new measuring tape must be prepared by cutting out or copying the "calories per ounce" scale at the top of the chart. For instance, to find the calorific value of 1 ounce of butter we measure one unit on the butter scale with our new tape and find it to be 217 calories, whereas the correct calculation gives us 217.38 calories.

If the weight of the food is recorded in grams, the same chart is used, but a third form of measuring tape is prepared by cutting out or copying the "calories per gram" scale at the top of the chart. For example, to find the calorific value of 1 gm of butter, we measure one unit on the butter scale with this third form of tape, and we find it to be 7.7 calories, whereas the correct calculation would give us 7.668 calories.

In case the food is not sold by weight, the capacity of containers may be estimated. The volumetric capacity of containers may vary if not fixed by law, and the gravimetric capacity may vary with variation in the character of its contents. The tabulated data, obtained in San Francisco, may be of some use. The weights are net.

ESTIMATED CAPACITY OF CONTAINERS

Apples	40 lbs. per box (135 apples in a 4-tier box)
Bananas	70 lbs. per bunch.
Beets	10 lbs. per dozen.
Cantaloupe	65 lbs. per crate
Cassaba melon	40 lbs. per crate.
Carrots	80 lbs. per sack
Cauliflower	40 lbs. per dozen
Celery	3 lbs. per bunch
Cucumbers	40 lbs. per lug box.
Corn (green)	70 lbs. per sack.
Eggs	54 lbs. per case, 1½ lbs. per dozen
Grapes	45 lbs. per lug-box, 40 lbs. per crate.
Grapefruit	65 lbs. per crate (64 grapefruits)
Lemons	70 lbs. per crate (350 lemons).
Lettuce	40 lbs. per crate, 13 lbs. per dozen
Milk	2 lbs. per quart.
Oranges	70 lbs. per crate.
Peaches	55 lbs. per lug-box, 40 lbs. per pony-crate.
Potatoes (sweet)	118 lbs. per box
Radishes	7 lbs. per dozen bunches.
Spinach	10 lbs. per dozen bunches.
Tomatoes	55 lbs. per lug-box.
Turnips	80 lbs. per sack.

The weight of contents of canned and dry package goods is marked on the container. The foods sold by volume and not weight are usually of low calorific value, and hence the absolute error in estimating the weight is very small.

*Reprinted from The Journal of the American Medical Association
Jan. 10, 1920, Vol 74, pp. 101 and 102*

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American Medical Association, 535 N. Dearborn St., Chicago

*Nutrition and Public Health, with Special
Reference to Vitamines*

BY

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FROM THE
AMERICAN JOURNAL OF THE MEDICAL SCIENCES
April, 1920, No. 4, vol. clix, p. 477

NUTRITION AND PUBLIC HEALTH WITH SPECIAL REFERENCE TO VITAMINES.¹

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NOTWITHSTANDING the fact that my first biological interest was in relation to the subject of nutrition, my earlier experiments were made on myself and were discontinued as soon as my curiosity was satisfied, and I have had occasion to work out, in a scientific manner, only small details of this vast subject. While serving with the Division of Food and Nutrition, Medical Department, U. S. Army, I was impressed with the wide differences of opinion that exist in the public mind, and especially among the medical profession, on this subject, and since January 1, 1919, with the assistance of ten students, I have devoted nearly all of my time to investigation in this field, the results of which are being published in the *Journal of Biological Chemistry*. Owing to the fact that the changed economic conditions due to the war have brought about some changes in dietary habits, the effects of which may not be thoroughly appreciated until some years have passed, a compilation of some of the literature and discussion of some of the problems may not be out of place at this time. Since vitamine studies are receiving considerable attention just now, attention will be directed especially to them throughout most of this paper. Other subjects will be treated more briefly, not because they are less important, but because they are better understood.

EFFECT OF FUEL VALUE OF DIET ON BODY WEIGHT. Benedict, Miles, Roth and Smith have so thoroughly investigated the effects of a restricted diet (about 2 ounces protein and 2000 calories per ration) that further comment seems unnecessary. Similar restrictions of diet have been voluntarily or involuntarily made by large numbers of persons during the war. One common conclusion from these experiments may be emphasized here, since I have heard it repeatedly denied by persons who had no reliable information on

¹ Published with permission of the Surgeon-General, U. S. Army.

the subject, and that is that the body weight is just as much dependent on the calorific value of the food in man as in animals. Perhaps many persons partake of a low calorific diet in the presence of an abundance of food, owing to various factors that reduce the appetite. In animal experiments a diet that is inadequate in regard to some constituent reduces the appetite. This has been especially noticed in experiments in which one or more of the amino-acids or vitamins was deficient in quantity. Animals on a vitamin-free diet finally refuse food entirely. This factor may be suggested as a possible danger in so-called low protein diets.

As early as 1887, Hirschfeld advocated a low-protein diet and the same ideas have been proposed by many scientific and unscientific propagandists since that date. In 1898, while a university student, subject to compulsory physical exercise, I restricted my diet for three months in regard to the quantity of animal foods taken. My weight dropped to 70 pounds and my skin became rough, the hair on my body and face standing at right angles to the skin surface. At the end of this experiment I partook of a liberal diet, including fresh meat and vegetables, eggs, milk, beef fat, cod-liver oil, raw bone-marrow and malt extract, and at the end of one year my weight was 140 pounds, showing a gain of 3 ounces per day. In order to distinguish between quality and quantity of the diet I made another experiment in 1912. After having maintained a fairly constant weight under certain conditions for several years, and without consciously disturbing those conditions, I added about 2 ounces of cottonseed oil per day to my diet and gained 3 ounces per day for eighty days. Since the oil was drunk after meals it did not interfere directly with my appetite, and since it is not known to contain a vitamin or other growth-producing substance, it probably acted purely through its calorific value or was stored, together with water, as adipose tissue. Since fat does not cause excessive water storage, as is the case with carbohydrate, the increase of about 15 pounds in weight cannot be attributed to water storage alone.

Although a restricted diet has been advocated by Horace Fletcher, Chittenden, and more recently by Hindhede, these propagandists seem to be in the minority. Another point of view is illustrated by the excuse a slave gave for stealing a chicken: "Nigger meat's wurf mor'n chicken meat." There are some uses of a restricted diet, however, but the difficulty lies in enforcing the dietary rules. It is said that in the treatment of obesity at Carlsbad the patients were served very small portions at high prices *à la carte*.

In economical rationing a rapid and inexpensive method of calculation of fuel value of food is desirable. By means of the chart shown, simultaneous multiplication and addition is performed in calculation of the calorific value of a list of foods. The time required is chiefly used in finding the name of the food on the chart, and hence

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speed increases with practice. The first time I used this chart the percentage error and the number of minutes required were carefully noted. The food used by the 9th Engineers in one week was used in the test. It required five minutes to calculate the calorific value of this food with the chart, and the error was 5 per cent. By taking more time to read the chart, or by using a chart on a larger scale, greater accuracy may be obtained in the calculation, but since the accuracy of the data may not exceed 5 per cent., greater accuracy in calculation may not be desired.

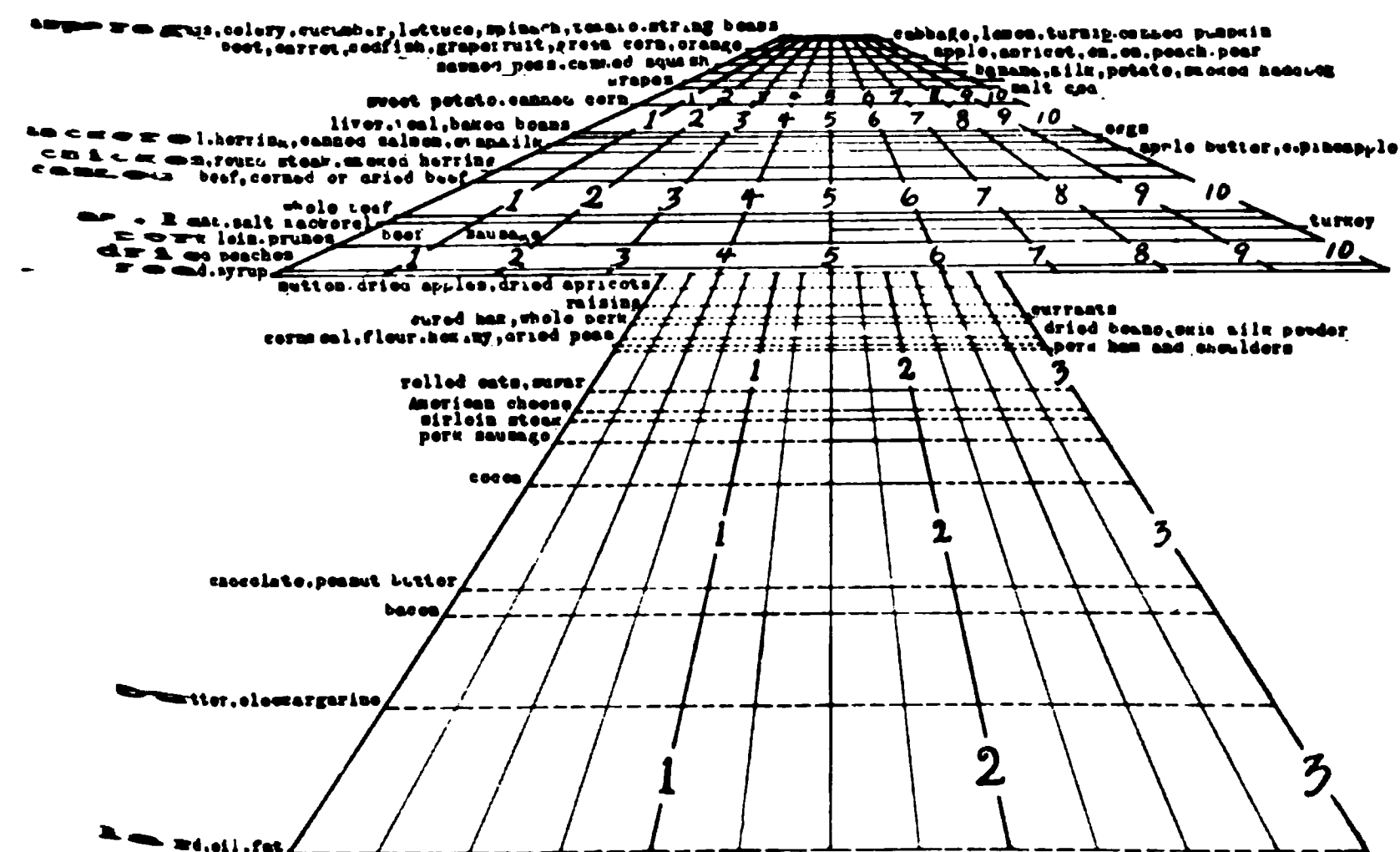


Chart for rapid calculation of fuel value of diet. The calorific value of any food be calculated from its weight in pounds by finding the name of the food in the chart and the number corresponding to its weight on the line containing its name. Apply a millimeter tape to the scale on the chart and each millimeter represents 100 calories. In the upper part of the chart the scale reads in pounds from one to ten. In the lower part of the chart the scale reads in pounds from one to three and further divisions signify halves and quarters of a pound and the breaks in the horizontal lines signify ounces. Foods of the same calorific value are on the same line and their weights may be added together before using the chart. In order to use the chart for simultaneous multiplication and addition, prepare the list of foods in pounds and measure the pound scale of each successively with the millimeter tape, commencing each measurement on the tape at the end of the preceding one. The final figure on the tape with two ciphers added represents the total number of calories of the entire list of foods.

REACTION AND BACTERIOLOGY OF THE ALIMENTARY TRACT. The reaction of the alimentary tract is important in relation to the character of the hydrolysis of the food, either due to digestive juices or bacteria. The saliva in the closed mouth is about neutral. The gastric juice is about one-tenth normal hydrochloric acid and the exact hydrogen ion concentration of the stomach contents depends on that of the food and the relative quantity of gastric juice that has been secreted (as well as the degree of mixing.)

McClendon and Sharp have shown that with the occasional exception of fresh milk, both raw and cooked foods are slightly acid. It should be noted that in the paper by McClendon and Sharp the first table (quoted from Clark and Lubs) should be omitted, since acid or alkali had been added to the food juices in many cases. The low acidity of the contents of the infant's stomach after feeding is not so much due to lower acidity of the gastric juice as to the diet of fresh milk, and possibly to the small quantity of gastric juice secreted. It was shown by McClendon, Myers, Culligan and Gydesen that the small intestine is slightly acid in young and adult carnivora and young herbivora. In adult herbivora, however, the duodenum is usually slightly acid, but this passes over to a slightly alkaline reaction somewhere in the small intestine. The reaction of the rabbit's intestine could be changed from alkaline to acid by flooding it with soluble carbohydrate, as by adding lactose to the diet or feeding carrots exclusively.

According to Metchnikoff the presence of acid-producing bacteria in the intestine inhibits the growth of putrefactive forms. Torrey has shown that flooding the intestine with soluble carbohydrate, especially lactose, increases the ratio of the acid-producing to the putrefactive bacteria. This is in line with the fact that the ingestion of soluble carbohydrate changes the reaction of the rabbit's intestine from alkaline to acid. Perhaps it is the acid reaction of the intestinal contents that inhibits the growth of putrefactive bacteria. Exercise, by causing more rapid absorption of water from the intestine, may decrease the growth of bacteria, whereas in diarrhea the increased fluidity increases bacterial growth.

Besides the common bacteria of the intestine just mentioned that are always present, and whose relative numbers depend on the soluble carbohydrates present, other bacteria that are normally absent appear as infections, as, for example, the typhoid and paratyphoid group. It seems possible, also, that other less virulent, unidentified bacteria may play a part in altering the normal conditions of nutrition.

COOKING. In *The Complete Cook*, 1805, which is the revised and corrected edition of *The Frugal Housewife*, the following sentence may be found in the preface: "The chief excellence of all cookery consists in a perfect acquaintance with the making of gravies and sauces." That is not the aspect of cooking to which reference will be made. The natural flavors of foods with the addition of salt, which is a necessary ingredient of the diet, should be sufficiently attractive to the hungry man. The necessity of cooking lies in two circumstances: (1) it is necessary to gelatinize the starch in starchy foods in order to make it utilizable and (2) it is desirable to kill certain pathogenic organisms or destroy bacterial toxins when present. Most starches gelatinize in water of 70° C. in a few moments, and prolonged cooking is only necessary in case the food

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is so dry that prolonged soaking is necessary to wet it (and the heat merely shortens the time necessary to soak it). Cooking softens food so that it is easier to chew, but that may not be wholly desirable. If it is desired to gelatinize collagen a high temperature or prolonged duration is necessary, but gelatin is too deficient a protein to be much sought after.

Many persons who would not eat a rare steak see no necessity in cooking canned goods. From the standpoint of danger from bacterial agencies, however, it is more necessary to sterilize preserved foods than fresh ones. Many cases of botulism are due to eating canned foods or tasting them to see whether they are spoiled, and it is stated that cooking destroys the toxin of *Bacillus botulinus*. Since cooking reduces the vitamin content of foods any unnecessary cooking is to be discouraged. Fresh foods if kept clean should be eaten raw or rare, whereas preserved foods, as a rule, should be cooked. Pork and fish should be sterilized because they are liable to contain parasites that escape detection. Mixtures of meat and vegetables should not be allowed to remain long uncooked, because numerous spore-bearing bacilli on the vegetables multiply rapidly in the meat juices.

Many persons object to visible fat in cooked food; in fact, some cooks remove fat from the surface of soup with blotting paper. Fried foods are said to upset delicate stomachs, and this has been attributed to the action of heat in setting free fatty acids. It has been shown, however, by the War Committee of the Royal Society that free fatty acids in relatively large quantities may be eaten without disagreeable effects. Perhaps the irritant action of some fried foods may be due to the formation of acrolein. Since this occurs only in case the temperature is raised to 300° C., there is no necessity for it. The fat of all foods should be eaten as an economical measure.

FOOD PRESERVATION. The most ancient and widely used method of food preservation is by desiccation. The seeds of plants are usually desiccated before they are gathered for food. The desiccation of root vegetables was practised by various savage tribes and the drying of meat and fish was perhaps as common a practice. Hawk calls attention to the fact that dehydrated vegetables when soaked up again hold their water much less tenaciously than fresh vegetables do. This has been known as a characteristic difference between live and dead tissue, and the living state is associated with electrical polarization of the cell surfaces, which would partially explain the holding of water. In fact, it is the tenacity with which foods hold to water that adds largely to the cost of dried foods. In the drying of prunes, decomposition usually takes place unless the prunes are first dipped in an alkaline solution, so as to make them permeable to water. In the desiccation of tissue for biochemical analyses it has long been known that decom-

position of phosphatides and oxidation of unsaturated fatty acids occurs if the moist or dried tissue is exposed to air, and similar decomposition must occur in dried foods.

Various forms of pickling and preserving in which the addition of salt or sugar occurs is similar to drying, in that the antiseptic action of salt and sugar is partially due to their dehydrating effect on bacteria. The use of other antiseptics is so limited as to be of minor importance.

The process of canning might be considered next to refrigeration in excellence as a method of preserving food, but unfortunately, complete sterilization and exclusion of air does not always occur. In the canning of milk, partial desiccation is also performed.

In the process of cold storage, vegetables and fruits remain alive, and it is claimed that fish may be kept alive in cold storage by the Pictet process. The growth of penicillium on beef in cold storage hastens ripening and the beef does not become toxic until the taste changes to musty or bitter. Beef is the best meat for cold storage on account of its large size and low water content and mechanical density, which inhibit the penetration of microorganisms. Cold storage is the only method of food preservation that maintains the total vitamine content.

PROTEINS, FATS, CARBOHYDRATES, SALTS AND WATER. The protein factor in nutrition has been so exhaustively treated by Osborne and Mendel and others that it cannot be reviewed here. As a general rule animal proteins are more complete in amino-acid content than are those of vegetable origin. According to Goldberger, Wheeler and Sydenstricker, families in which pellagra occurred consumed 3 ounces of protein per man per day, but only 25 per cent. of it was animal protein. Since a nitrogen balance may be maintained in skinny individuals on less than 3 ounces of protein per day, we may assume that 3 ounces is sufficient, provided it is all animal protein; but that in case of mixed proteins, and in the absence of more definite knowledge, it is not wise to make a special effort to limit the protein intake. Under modern conditions there seems to be no defence for vegetarianism. Clark finds a high protein diet increases the rate of healing of wounds. It is also useful in case of profuse pus formation to replace the lost protein.

In addition to the fact that some fats contain vitamins, fats are necessary in the diet to decrease its bulk. In a diet of 3000 calories it would require 10 pounds of potatoes or other vegetables or fruits for the three meals, and if we include the saliva and other fluid swallowed and the gastric juice secreted the stomach would receive a load of about 8 pounds per meal (not all at once, however). Hardtack would be less bulky, but would increase salivation and thirst. The Interallied Scientific Food Commission fixed the minimum fat ration at 75 grams.

So far as investigations have progressed, muscular work depends

on a supply of carbohydrate (compare Anderson and Lusk). In the absence of carbohydrate, fat is incompletely oxidized (for heat production) and yields products that may be toxic. Both man and the dog may live for considerable periods without carbohydrate in the food and probably depend for muscular energy on the carbohydrate radicle of phosphatides and glycoproteins and especially on the synthesis of glucose from glycerin and deaminized amino-acids; but there seems to be no advantage in such a diet. The flooding of the intestine with soluble carbohydrate retards putrefaction of proteins.

Salts are necessary, but since all factors causing their excretion have not been thoroughly investigated the minimum requirement is uncertain. Sodium chloride should be added to food, but it seems probable that the other salts are present in a mixed diet in sufficient quantity. Since milk and fresh vegetables that are rich in salts are also rich in vitamins that cause relative retention of salts, all that can be said is that milk and fresh vegetables are advantageous, and we are not justified in substituting an inorganic salt mixture for them. No harm can arise, however, in the addition of such a mixture to an apparently adequate diet.

The necessity of water in the diet needs no comment, and Hawk and his collaborators have shown that no ill-effects follow water-drinking at meals. The salt content of drinking water is usually insignificant, and the arguments for lithia water have been shown to be fallacious.

VITAMINES. Owing to the fact that differences of opinion exist among investigators as to the number of vitamins, the classification made by the British Committee on Accessory Food Factors will be followed. Hopkins studied "Accessory factors in food." Funk called these vitamins, and McCollum and Kennedy claim there are only two vitamins, which they call fat-soluble-A and water-soluble-B. Drummond refers to the antiscorbutic factor as water-soluble-C. McCollum and Kennedy's A-factor is also called fat-soluble or antirachitic vitamin, and their B-factor is called antineuritic vitamin, whereas Drummond's C-factor is usually called antiscorbutic vitamin.

The fat-soluble or antirachitic vitamin is very abundant in yellow fats of animal origin. It is deficient in pig fat because the pig eats few leaves, and in hydrogenated or hardened fats and free fatty acids because it is destroyed by the heat and chemical agents used in manufacture. It is deficient in vegetable fats except perhaps in peanut oil. It is synthesized in green leaves, and carnivorous animals depend for their supply on the bodies of grazing animals in which it has been extracted from the green leaves eaten and stored in the body fats. It is abundant in the germ of seeds, especially yellow seeds but deficient in degerminated cereals. It is present in potatoes, carrots and bananas, being most abundant in the

yellow colored roots (carrots and sweet potatoes). According to McCollum the milk of cows is richer in fat-soluble-A when more is present in the cow's food. According to Osborne and Mendel, 1916, this vitamine is not destroyed in butter fat by storage for a year. According to Drummond, fat-soluble-A is not carotin.

The antineuritic vitamine seems to be most concentrated in the seeds of plants and eggs of animals. In cereals it is present in the germ but absent in the endosperm and bran (unless the bran contains the germ. It is abundant in yeast and in animal gland tissue, but muscle tissue and milk do not contain an oversupply. Both leaf and root vegetables and fruits contain it in small amounts. Cooking reduces the quantity of this vitamine. As first observed by Frazier and Stanton the content of food products from the same source in this vitamine runs parallel to the phosphorus content. According to Voegtlin and Myers, both antineuritic and fat-soluble vitamins of wheat and corn products run parallel to the phosphorus content.

The antiscorbutic vitamine seems to be present in all living tissue and fresh food, and is especially abundant in acid fruits, tomatoes, cabbage and turnips. It is more or less abundant in all fresh, raw vegetables. It is very sensitive to alkali, heat, desiccation and storage at ordinary temperatures, and hence is deficient in cereals and dried or canned meat and vegetables, except canned tomatoes. The acid in tomatoes and fruits seems to protect it during the heating and it is useful to class tomatoes with fruits, as is done by British writers. It is present in milk in small amounts. Dutcher has shown that young guinea-pigs grow better and are healthier on milk from a cow on green feed than of a cow on dry feed. Scurvy developed very quickly on winter milk, but did not develop (in the same length of time) on summer milk. The milk was skimmed in both cases to prevent diarrhea. This indicates that the antiscorbutic vitamine in milk depends on that in the cows' feed, and may explain why some breast-fed babies have scurvy if the food of the mother is deficient in antiscorbutics.

VITAMINE REQUIREMENTS AND DEFICIENCY DISEASES. Since the composition of vitamins is unknown and their distribution among foodstuffs is known only in a general way, it is not yet possible to work out the vitamine requirements with even as much certainty as has been done in the case of food constituents of known composition. Experiments on animals and observations on man indicate that vitamine deficiency manifests itself more slowly the longer the average life (in different species) and the more mature the individual (in the same species). It is also necessary to know that the individual has been getting an adequate supply of vitamins before the beginning of the experiment in order to determine the duration of vitamine deficiency that it can stand. Even with this precaution, variations in results may be obtained owing to lack of

knowledge of the degree of vitamine deficiency in the food. At present the vitamine content of foods can only be determined by the quantity of the food necessary to prevent or cure deficiency diseases. Hence the vitamine requirement may be estimated only in terms of the proportion of vitamine-containing foods required in the diet.

That the deficiency diseases are due to lack of vitamins can be determined only by the method of exclusion. Almost every imaginable cause has been attributed to the deficiency diseases, and some causes have been proposed that it is impossible for me to imagine. For instance, scurvy has been attributed to lack of ionization of the salts in the food due to staleness, and it is impossible for me to imagine a lack of ionization of these salts in food that has been eaten, and certainly food will not hurt you if you do not eat it. Scurvy has been attributed to an acid-forming diet, but McClendon, Cole, Engstrand and Middlekauff have shown that this factor is not responsible for scurvy. The germ theory of these diseases will probably be popular for a long time, since bacteria are always present, waiting for an opportunity to hasten the death of a weakened individual. It is therefore useful for purposes of discussion to include among deficiency diseases those that have been cured in the presence of a vitamin-rich diet and have not been cured in the absence of such a diet. We will consider deficiency diseases of infants and adults.

Rickets is perhaps the most common deficiency disease, frequently occurring in breast-fed infants. To quote a book written before Funk's ideas of vitamins were generally known, Miller states that, without doubt, rickets is to be regarded as a dietetic disease, and considers deficiency of butter fat as the most constant and potent cause. In treatment, among other things, he recommends milk, cream, butter, egg yolk and cod-liver oil. These fats are especially rich in the antirachitic vitamine. Since the bones are softened in rickets the child may not be able to take proper exercise or go out into the fresh air, hence the view that rickets is due to lack of exercise or fresh air. The mother's milk might be sufficient in antirachitic vitamine, and yet the child may have rickets, due to the fact that it was weaned too soon. The Fijians, who included some of the finest specimens of physical development in the human race, nursed their infants for three years, and as the mother's milk became deficient in quantity, supplemented it with bananas. Rarely a mother in this country nurses her children for three years, but in savage Fiji it was a religious custom, and the taboo was placed on the mother in order to prevent the interference of the father. The prevalence of rickets among negroes may be due to the fact that the mother's diet of corn bread and fat pork is deficient in antirachitic vitamine. The British Committee base their statements concerning rickets on the experiments of Mellanby on pups.

Hess and Unger failed to cure rickety babies on cream, but did so on cod-liver oil.

Xerophthalmia, keratomalacia or *xerosis conjunctivæ infantum*, an eye disease of infants that has been especially prevalent in the Scandinavian and other European countries during the war, is due to lack of antirachitic vitamine, the deficiency probably being greater than that required to produce rickets. It is accompanied by diarrhea. Sztark cites a case that developed after an exclusive diet of pea soup for fifteen months, and was cured in ten days on a diet of cows' milk.

Infantile beriberi occurs in infants nursing from mothers who have the disease, and whose milk is consequently deficient in antineuritic vitamine.

Infantile scurvy is due to deficiency of antiscorbutic vitamine. To quote again from "previtamine literature" Miller states that the opinion most commonly held is that scurvy is caused by a diet which through sterilization has been deprived of its "fresh element" and recommends orange and grape juice in its treatment. The fact that scurvy may occur in breast-fed infants, indicates variations in the antiscorbutic content of milk.

Marasmus is often considered a dietetic disease. Miller states that not seldom the child has thrived on the breast for a month or two, and has then been weaned in order that the mother may go out to work, and from that time has gradually wasted. Eddy and Roper claim that the addition of a powder rich in vitamins to the cereal food of marasmic babies increased tissue growth. Vitamins are certainly necessary for growth, but so are other food constituents.

Since the question of growth has been referred to, perhaps it is well to remember that for normal growth there is necessary not only vitamins, but also the secretions of ductless glands. It is well-known that the size of the ductless glands is greatly affected in deficiency diseases in man and animals. According to McCarrison, deficiency of vitamins in the diet leads to abnormalities in the secretion of the ductless glands. We know that iodine in the food is necessary for the normal functioning of the thyroid, and it is possible to imagine unknown constituents of the diet that may be necessary for the ductless glands.

Pellagra may occur in breast-fed infants whose mothers have the disease, but it usually takes a long time to develop.

Deficiency diseases of adults may be different from those of infants, because growth is complete, although many of these diseases characteristic of adults occur also during adolescence or infancy.

War edema, also called famine edema or hunger edema, has been ascribed to lack of the antirachitic or fat-soluble vitamine, or other element in the diet. The fat content of the diet in war edema

is not accurately known, but Guillermin and Guyot state that the total ration was sometimes as low as 800 calories, and it contained a very small proportion of fat, and that a decrease of 40 per cent. in body weight was common. Park has shown that it is readily cured by a return to the normal diet. War edema, or famine edema, as it is more often called, is not a new disease. Lusk refers to an account of it in France in 1817, just 100 years previous to the late outbreak in Germany and other European countries and Mexico. He also states that cases were cured in a week by the addition of 100 grams of fat to the ration. It is stated that edema occurs when there is a decrease in blood-plasma proteins, and hence that decrease in protein in the diet may cause edema. A diet of 800 calories fed prisoners is naturally deficient in protein. The scorbutic symptoms observed by Beyerman in famine edema suggest scurvy or a complication of scurvy. He states that the edema was cured by adding vegetables to the diet. Edema occurs in various deficiency diseases, and hence some confusion may arise in the classification, but the large number of cases of war edema that have occurred recently, indicate that it is a separate disease. Schittenhelm and Schlecht observed decrease in blood proteins in war edema.

Beriberi is a form of multiple peripheral neuritis caused by a deficiency of antineuritic vitamine, and is most prevalent in rice-eating countries. Wet beriberi is accompanied by edema, but in dry beriberi this is substituted by atrophy. As is often the case in deficiency diseases, there may be gastro-intestinal disturbances. The principal cause is too exclusive a diet of polished rice, and according to Fraser and Stanton, the substitution of undermilled rice, containing at least 0.4 per cent. of phosphorus, prevents the disease. It may be caused by too free use of any degerminated cereal. The addition of canned goods to the diet may not prevent it. There is apparently not enough yeast in bread to prevent beriberi. Beans and peas are perhaps the most useful preventives. In the treatment of the disease, extract of rice-polishings and fresh yeast or autolyzed yeast have been used. Funk, Lyle, McCoskey, Caspe and Poklop observed a mild glycosuria in man on a diet of white bread and polished rice.

Scurvy was perhaps the first deficiency disease to be recognized as such. Captain Cook showed that it could be prevented by the use of fresh vegetables. Nansen and Johansen passed a winter on an exclusive meat and fat diet without scurvy. Stefansson claims to have cured cases of scurvy by a raw meat diet. Apparently all fresh foods have traces of antiscorbutic vitamine. The juice of sweet limes or lemons is especially rich in this vitamine, and this fact has led to the development of a large industry for bottling lime juice. This preparation was not tested until recently, and was then found to be deficient. By some mistake, sour limes

have been substituted for the curative sweet limes, and the lime juice of commerce does not come up to expectations.

Pellagra was called alpine scurvy by Italians, and has been shown by Goldberger to be a deficiency disease. By definition the disease is characterized by a symmetrical dermatitis, especially of those parts exposed to the sun, but this is not its most serious characteristic. The worst features of beriberi, scurvy and pellagra are the effects on the nervous system. The hemorrhages in pellagra are in the nervous system, whereas in scurvy they are general (and may occur in the meninges). Sundwall has shown that the tissue changes in pellagra are not specific, but are similar to those in animals on inadequate diets. In beriberi, pellagra and scurvy gastro-intestinal disorders are present. In pellagra edema is extremely localized, in scurvy it is associated with hemorrhage and in wet beriberi it is more general. Pellagra is of very wide distribution and may be worldwide. Since a patient with pellagra may live ten years, it is probably not due to complete absence of any dietetic constituent but to a slight deficiency. Whether this is a vitamine or an amino-acid, or both, has not been determined.

Trench foot is attributed by Bruntz and Spillmann to lack of vitamines associated with life in the trenches, the predisposing cause being the diet, and the water-filled trenches causing the acute symptoms in the feet.

Sprue was classed as a deficiency disease by Cantlie, who found scurvy in some cases. Brown states: "In the open alluvial workings of a tin mine in the East Indies all of the employes are exposed to the same atmospheric conditions. The Chinese coolie is stricken with beriberi, his European overseer with sprue. In no essential condition, even in that of diet, are the circumstances of their daily life materially different. The treatment of sprue is essentially dietary. A milk diet, a raw fruit diet, a milk and fruit diet or a raw or rare meat diet is used. In any case the diet contains vitamines."

VITAMINES AND APPETITE. In all animal experiments on a vitamine-deficient diet loss of appetite occurs. With human beings, psychological factors may enter in so as to make it difficult to perform clean-cut experiments on this subject, yet the final loss in tissue substance in all deficiency diseases suggests that the appetite has been affected. We know that pure proteins, fats and starches have no taste, but we do not know whether vitamines have flavor. We do know, however, that the taste of milk changes on boiling and the taste of many foods changes by canning or drying. Why is it that some persons have been known to defy the cholera germ for a raw oyster and the typhoid germ for a salad, and to give their lives for a momentary tickling of the palate? There must be some great advantage in fresh foods or else the desire for them would be eliminated by natural selection. To the clothes-moth the age of food seems to make little difference, but when a man sits down to

a meal of storage eggs, canned milk, jerked beef and hardtack it requires considerable hunger to whet the appetite. This craving for fresh food has probably saved more lives than are killed by germs. The human race has existed for some time prior to the advent of food chemistry, and most people live nowadays without the aid of that science. Unquestionably the appetite has played some part in the preservation of the race. It seems probable that the person who is presented with an adequate choice of natural foods from infancy will choose an adequate diet, and that the cause of the deficiency diseases is that an adequate choice of foods is not always supplied to the table. In the case of animals this question has been tested. Evvard allowed pigs free choice among a variety of foods, and in this way produced the largest pig for its age that was ever raised at the Iowa Experiment Station. A more rigorous experiment was made by Osborne and Mendel, since they did not use natural foods but purified components made into a biscuit. A rat presented with a more and a less adequate biscuit might eat for a few days from the less adequate, but finally showed preference to the more adequate diet. These experiments did not refer to vitamins exclusively, but they show that an inadequate diet results in some change in the appetite.

FOOD SUBSTITUTION. According to Steenbock, fat-soluble-A is more abundant in yellow foods than in corresponding white ones. Yellow corn contained more than white corn, yellow oleo oil more than white fat separated from the same adipose tissue, yellow sweet potatoes more than white potatoes. The artificial coloring of butter may therefore deceive us as to its vitamin content. Without doubt the operation of the Pure Food and Drugs Act and the work of the laboratories operating under this law have been of inestimable advantage to the public. Food chemistry has not reached a sufficient stage of development to be relied on exclusively in questions of food substitution. Feeding tests are necessary, and the preference of the public for natural products, on which feeding tests have been made by the human race, is well grounded. The work of food fakirs has been insidious. Take the question of oleomargarin. The early products coming under this label in this country contained a large percentage of oleo oil, which is rich in the fat-soluble vitamin. Little by little the proportion of such oleomargarins has decreased and perhaps the majority of oleomargarins today are made from cottonseed and cocoanut oils and are deficient in fat-soluble vitamin. Galicians eat lard on their bread, but it is said that the black bread they use contains some fat-soluble vitamin from the rye germ. Negroes have a particular fondness for pork fat and white cornmeal bread, and this may be the chief cause of the prevalence of rickets in negro babies, since the diet of the mother does not contain enough fat-soluble vitamin to be transmitted to the milk. In a milk-canning factory in this country,

skimmed milk and vegetable oils were run through a homogenizer and canned as milk compound. The cook may see the label on the artificial milk or butter, but the defenceless boarder does not even have the satisfaction of reading his sentence. Another deception is the soaking of dried green peas in water and then canning them. The Pure Food and Drugs Act does not allow the picture of a pea-vine to be placed on these cans, but the average consumer is not familiar with such distinctions in labels.

In natural foods a sweet taste is associated with fruits that are rich in antiscorbutic vitamins. If we may suppose that the taste for sweets is inherited, our savage ancestors may have eaten raw fruits, just as the monkeys in the jungle do nowadays. Most of the sweet food and drinks of modern man are made from sugars and molasses and honey that are free from vitamins. Coffee and its substitutes and tea, malted or distilled liquors, wines and carbonated drinks with synthetic flavors are deficient in vitamins.

I ordered a meal in a "pure food restaurant" and received various fake dishes, including an imitation beefsteak made of degerminated cereals. The fact that we use milk with cereals is probably what saves us from disaster. One is reminded of the old story of the soup-stone: A man knocked at the kitchen door and said he was introducing soup-stones. The stone was to be boiled in water, and he demonstrated it to the housewife, suggesting various ingredients that might be added to it. These were promptly produced by the enthusiastic housewife and the man drank the soup, leaving her the stone. Cereals form the basis of our diet, but scurvy develops when they are used as substitutes for potatoes or other vegetables, as occurred in Glasgow, Newcastle and other cities during a potato shortage during the war. In one institution in the United States there were 200 cases of scurvy on April 1, 1916, following two months' reduction of the potato and other vegetable ration to one-half.

THE MILK QUESTION. The rise in the cost of living, more than the rise in the cost of milk in particular, has led to a reduction in the consumption of milk by families with small incomes. Harris records data from 2084 families, representing in nationality and income a typical cross-section of New York City in the summer of 1918. In 14 per cent. of these families milk was entirely eliminated from the children's dietary. McCollum has repeatedly emphasized the advantage of milk in the diet. Mendenhall states that every child from eighteen months to twelve years of age is better for having one and a half pints of milk in its daily diet, and that fluctuations in the demand for milk or diminished use of milk throughout the country will inevitably result in a lessened production, and that the loss of our herds at this critical period would be a calamity. Hart and Steenbock call attention to the use of milk as a supplement to cereal foods. Milk makes up the deficiency of cereals in vitamins,

salts and certain amino-acids of the proteins. The United States Food Administration calls attention to the fact that milk compares favorably with other animal foods in price per gram of protein and in price per calorie, and that there is only enough milk consumed in this country to give each person a little more than half a pint per day. Twenty and more years ago a professor of mathematics used to tell a story of being hard up in his college days. He calculated that he would have ten cents a day to spend for food, and that the highest nutritive value could be gotten from ten cents' worth of milk. Practical experience, however, suggested a change to five cents' worth of milk for nourishment and five cents' worth of mince pie to "stay with" him.

The chief value of milk lies in the fact that it is a complete food and does not require any balancing of components or any precautions to include the minimum requirement of any constituent. I have never found a species of cooks who would calculate or weigh or balance any food constituents except under immediate compulsion. With a milk diet for the infant and a modicum of milk in the diet at all other ages the expense of calculation of dietary constituents is eliminated, and milk even at a high price may be worth buying in order to avert the danger of malnutrition and doctors' bills or loss of efficiency. It is an open question, however, whether cows' milk has been beneficial to infants in general. During the famine resulting from the siege of Paris the infant mortality fell 40 per cent., due to the fact that mothers nursed their own infants. A similar decrease occurred during the great Lancaster cotton famine, due to the fact that the mothers could not work in the factories and did not have the money to buy cows' milk for the infants. During the recent occupation of Lille by the Germans, when the adult population lost about 40 per cent. in weight, there was a reduction in infant mortality, due to the fact that the mothers nursed their own infants. In the absence of mothers' milk, however, cows' milk is the best food for infants, but it is better to feed the cows' milk to the mother if she can nurse her child.

ARMY RATIONING. It seems probable that the chief point of attack of faulty nutrition on the military strength of a nation is on the infant males. Crooked bones, bad teeth and other deficiencies may result from faulty feeding of prospective soldiers. If the soldier going into action has a well-nourished body to begin with a short campaign on very defective rations may not lead to serious consequences. The protein, fat, mineral salts and vitamins stored in the body may be drawn on to make up the deficiency. It is often stated that the body cannot store protein, and it is true that protein is not stored in the same way that fat is; but the protein of the muscles may be drawn on for a considerable period, as has been the case in millions of persons during this war.

The ration allowed the army by Congress has been subject to

many variations. The soldier eats what he can get, but must necessarily be influenced by the ration allowance. During the Revolutionary War conditions were very variable. In 1780 the American Army was increased by the addition of the French Division (5000 men), who landed from transports with over 600 cases of scurvy among the soldiers and 1000 cases among the sailors after a voyage of two months and nine days.

In 1775 fresh milk was included in the army ration, but since it could not always be supplied a revision of the ration was soon necessary. In 1798 Congress fixed the ration at 20 ounces of beef, 18 ounces of bread and an allowance of salt, soap and candles. Food profiteers made a liberal interpretation of the words "bread and beef," so that the Surgeon-General thought it necessary to report unfavorably on the results. The two staple articles of the ration have remained practically unaltered in quantity until the present day, but later Congresses allowed liquor and specified substitutes for beef. In 1818 the President was authorized to make changes in the ration other than increase in cost, and in 1834 he substituted coffee and sugar for the liquor component. Temporary increases for the period of the Civil War were made, but except when raids were made on food stores the nutrition of the troops was not ideal. Owing to the large proportion of preserved food, including flour, salt meat, desiccated vegetables, canned milk and coffee extract, 30,741 cases of scurvy were recorded among white troops. In 1890 a pound of fresh vegetables was added to the ration and in 1908 the present garrison ration was authorized, which is of sufficient cost and allows sufficient substitution to furnish an adequate diet. The savings privilege in rationing in garrison, authorized in 1910, was abused to such an extent that it was discontinued April 1, 1919, and all substitutes are purchased through the quartermaster and the savings revert to the Government.

About the only data we have of the nutritive balance of our soldiers are their weight records. According to Munson the average peace-time recruit gains about 2.8 pounds in the first three and a half months of military service. Similar figures for the National Army, compiled by Hildebrandt, show a gain of 6.4 pounds in the first four months of military service. The total gain during the war has been reported as twelve pounds per man. Such figures are encouraging, considering the fact that some of the recruits were undernourished at the time of enlistment. If the added weight is not necessary for muscular strength it is useful in raising the resistance to disease and famine. The army has experimented with an emergency ration, consisting of a dried mass of chocolate, eggs, milk and sugar that has a high protein and calorific value, but no one has been able to eat it for more than three successive days. When water only is available a reduction of a pound a day in body weight occurs in warm weather and more as the weather turns

colder, and a few pounds excess body weight form the best emergency ration, since accidental loss is eliminated. Owing to the fact, however, that carbohydrate is stored to a very limited extent in the body, the use of hardtack, if available, is advantageous.

I have had opportunity to compute the protein, fat, carbohydrate and total calories per man per day in a large number of army messes and have studied the data compiled by the Food Division, and in the majority of the few cases of deficiency noted, have found that the mess sergeant was inaccurate in his accounts or was trying to produce an impression on the inspectors. In some cases the soldiers and their mess officers complained of the food. The chief ground of complaint was that there was not a sufficiency of fresh fruit and vegetables. I computed the mineral constituents, and especially the acid-base balance, of these messes, and found them usually acid. Certain foods, however, which yield a basic ash (dried beans, for instance) were served too frequently to please the soldiers. It was not the character of the ash of the food which influenced their comment, but its freshness. Certain national guard companies had company funds sent them from their home towns. I found some of these companies at Camp Cody, N. M., spending thirty cents per man per day for fresh milk and a more variable sum for fresh California fruit. A certain officer having about 2200 men in his command found inefficiency in one or more of his mess sergeants and decided to run the messes himself. He made up bills of fare for twenty-one meals and ordered all mess sergeants in his command to use them. The foods listed were those carried by the quartermaster and there was a conspicuous absence of the fresh fruits and vegetables of the local markets. I found the order to use the menu evaded more frequently the farther the mess was from headquarters, and at the end of ten days the scheme was abandoned. If the desires of the soldiers may be expressed in chemical terms, they desired vitamins, and especially antiscorbutic vitamins.

Whereas the ration allowance for soldiers on duty has been based on the price of food, and the same was originally allowed for sick soldiers, during a certain period each base hospital was allowed a fixed sum of money for each sick soldier on its rolls. On June 1, 1918, the ration allowance for a sick soldier was fixed at fifty cents. In some of the larger hospitals savings were made, but the smaller hospitals reported that the allowance was not sufficient. I have no records for the same hospital covering a long period, but by combining data from three hospitals some comparisons may be made. These data are from Camp Pike, Ark., June, 1918, Camp Cody, N. M., August, 1918, and Camp Fremont, Cal., November, 1918. During this period there was a general rise in the cost of food, and to meet this, reductions in the higher priced articles of food were made. In June 13 ounces of fresh meat per ration were served, in

August 7 ounces and in November only 3.2 ounces. In June 2.5 eggs per ration were served, in August 1.2 and in November 0.4. At Camp Fremont from August 1 to September 1 the rise in price of fresh eggs was from 52 to 89 cents per dozen, of fresh milk from 23 to 40 cents a gallon and of oranges from 6 to 13 dollars a crate. At the end of this period the fixed allowance was superseded by a sliding scale system based on the local cost of the garrison ration plus a certain percentage that was greater the smaller the hospital.

The above remarks apply to the army in this country, but the United States troops in other countries have not always fared so well. Beriberi among the Philippine scouts gave occasion for an order that the meat ration was to be eaten and that the rice was to be undermilled (Chamberlain). Riddell, Smith and Gutierrez reported 60 cases of beriberi in the United States Army in Porto Rico November, 1918. Potatoes, beans and canned meats and vegetables were supplied the troops, and these data led to the supposition that the reduction in vitamine by canning may have been a factor.

The fly problem is serious in the army. Ball has shown that the house-fly may be carried 100 miles by the wind. I have seen squads of soldiers swatting flies in mess halls when the civil authorities allowed a mountain of horse manure to remain two miles outside of camp.

DISCUSSION. Observations, some of which are reported in this paper, have led me to believe that the nutrition of some individuals, especially infants, is not ideal, and that the high cost of living is leading to worse nutrition. A large part of the population has unconsciously depended on the presence of milk in an otherwise inadequate diet, and the decrease in milk consumption that is now taking place is to be viewed with alarm. The supply of fresh green vegetables is not sufficient to be a substitute for milk. Grass is not adapted to human alimentation except in the form of sprouted grass seeds. In the sprouting of seeds vitamins are synthesized in the young leaves and a quick crop of vitamins may be obtained without the necessity of planting the seeds in the ground. McClendon, Cole, Engstrand and Middlekauff have most recently studied the question of sprouting cereal grains. Wheat or rye, sprouted until the shoot extends an inch beyond the grain and heated in water to 70° to gelatinize the starch, forms a cheap and convenient and palatable source of vitamins. The seeds may be freed from bacteria before sprouting (Braun and Duggar and Davis). Since beef fat is about as valuable a source of vitamine as butter is, beef drippings and fat should be eaten rather than thrown in the garbage can, and the same applies to some other animal fats. Prolonged cooking of fresh foods should be discouraged, but all canned goods should be heated to boiling before serving, in order to destroy toxins of *Bacillus botulinus* that might be present, unless a competent inspection of the goods has been made.

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Monthly. Illustrated. 1920 pages yearly. Price, \$5.00 per annum

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Are Iodides Foods?

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as been considered by some biologists chemists that living matter originated in and the elements of living matter correspond to those found in the sea water. We look, therefore, to the composition of matter for the elements we should expect to find in living matter. Sea water consists of H_2O and sodium chloride, and besides those the chief ingredients are magnesium, calcium, potassium and carbonates, sulphates and bromides, but there are also present the following elements in traces: ammonia, sodium, rubidium, caesium, strontium, barium, manganese, zinc, iron, cobalt, nickel, lead, silver, gold, radium, fluorine, iodine, phosphorus, phosphate, silicate, aluminium, boron and arsenic. In searching for these substances in living tissue they have been found chiefly in the organs of organisms. However, chemists are turning them to a greater and greater extent in the study of mammals. Damiens¹ finds bromine in a large number of animals and Gautier² finds iodine in quite a number of animals. We are familiar with the fact that fluorine is a normal constituent of bones and teeth and of the thyroid gland. In experiments on the nutrition of animals, I have found it convenient to feed them evaporated sea water and in this way insure a supply of all the necessary elements. Cameron and Carmichael³ have not observed any deleterious effect in giving rather large doses of sodium iodide to rats and rabbits. The use of sodium iodide in preventing goiter in sheep and in preventing the hairless pig malady is quite well known. The use of iodide in the treatment of goiter was first brought out by the work of Dumas, who was born in 1800 and studied pharmacy in Geneva. Dumas and Coindet found that iodine was valuable in the treatment of goiter. The use of sodium iodide in the prevention and cure of goiter was strikingly emphasized in 1917 by Marine and Kimball.⁴ This leads to the natural conclusion that the cause of goiter, or at least one of the causes, might be the lack of iodine in our diet. Iodine seems to be very rare in food and soils (Private communication of Oswald Schreiner) or else the former methods of detection have not been sufficient for such traces as do exist (See Kendall and Richardson⁵ for later methods). Iodine has been found in a number of rocks such as slates (Gentile⁶), limestones (Lembert⁷), dolomite (Rivier and Fellenberg⁸) and granites (Gautier) in Europe and has been reported in vapor from Vesuvius (Matteucci⁹), but it seems to be leached out so rapidly from soils it is seldom to be detected. Forbes¹⁰ failed to find iodine in about half of the specimens of foods, and Cameron¹¹ had a

micers, A., *Comptes Rendus*, 1920, clvvi: 930.
Damien, A., *Bull. Soc. Chem. Biol.*, 1921, i: 66.
Gautier, A., *Comptes Rendus*, 1920, clxx: 261; clxxix: 66.
Cameron and Carmichael, J., *Journal of Biological Chemistry*, 1920, xlv: 69.

⁴ Marine, D., and Kimball, O. P., *Jour. of Lab. and Clinical Med.*, 1917, iii: 40.

⁵ Kendall, E. C., and Richardson, F. S., *Journal of Biological Chemistry*, 1921, xliii: 161.

⁶ Gentile, 1849, *Jahresber. d. Chemie*, 251.

⁷ Lembert, 1851, *Jahresber. d. Chemie*, 319; *Jl. Pharm.* (3), xiv, 240.

⁸ Rivier and Fellenberg, 1853, *Jahresber. d. Chemie*, 924.

⁹ Matteucci, 1899, *Comptes Rendus*, cxxix, 65.

¹⁰ Forbes, E. B., *Bull. Ohio Agri. Station*, No. 299, page 487.

¹¹ Cameron, A. T., *Journal of Biological Chemistry*, xviii: 335.

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ailar experience. The question of the relation of goiter to locality has caused much discussion and most persons have come to the conclusion that goiter is due to the presence of some substance rather than the absence, but so much fruitless work has been done in an attempt to find this substance it would be well to investigate more thoroughly the question of the absence of some substance.

Goiter occurs largely in mountainous regions far from the sea. Iodine is so rapidly leached out of the soil that the supply of it may depend upon salt spray blown from the sea. During storms the waves are broken into spray and the water evaporated and the salt carried for long distances through the air. This salt is washed down out of the air by rains and contaminates the rain water. In the accompanying figure 1 taken from Emmons¹²



FIG. 1

shown a map of the eastern states, indicating the relative amount of sea salt in the rain water. Determinations were made by the weight of a certain constituent (the chlorine) by the ordinary silver nitrate titration, and sea water is of very uniform composition regard to everything except H_2O . That is to say, when the salts are diluted or concentrated, they are all changed in the same ratio, and the dry salt would be of uniform composition, so that the chlorine titration would indicate the relative amount of iodine. Evaporated sea water contains 50 parts per million of iodine, whereas the chlorine forms 55 percent of the evaporated sea water. The lines

¹² Emmons, W. H., 1913, *U. S. Geol. Survey Bull.*, 529.

on the map indicate parts per million of chlorine in the rain water and the iodine would be about one ten-thousandth of this amount, or, in other words, a part per million of chlorine would be about a part per ten billion of iodine. We can say, therefore, that the amount of iodine in the rain water rapidly decreases as we go from the coast, and is least in the Great Lakes region. Figure 2 (taken from Davenport and Love¹³) shows a map of

GOITER, SIMPLE

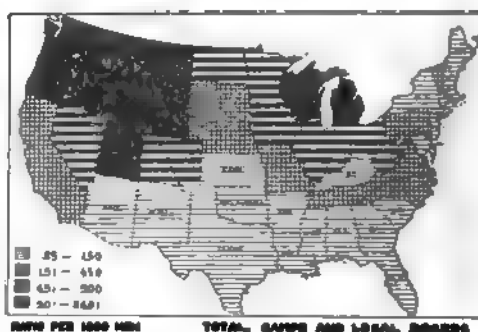


FIG. 2

the goiter as reported by the Draft Board and we have more or less the same distribution in the opposite direction and see more goiter towards the lake region and less toward the coast. Owing to the fact that no chlorine maps have been made for the rest of the country, it is not possible to extend this comparison. It is reported, however, from various sources (and is my personal observation in Minnesota) that the whole Great Lakes region is quite goiterous, and this is necessarily a region in which very little sea salt falls upon the land since the air blowing over it has already been washed free from sea salt by previous rains. Besides this goiterous region, various mountainous regions in the country have been reported to be goiterous and this is also true of Europe. These mountainous regions may be relatively near or far in relation to the sea. We often speak, however, of the clear mountain air free from dust, and it seems very probable that sea salt, being very heavy, would tend to remain in the lower strata of air rather than rise to mountain

¹³ Davenport, C. B., and Love, A. G., 1920. *Scientific Monthly*.

Volcanic dust when thrown to great heights may remain in the upper air for a considerable time, but this is true only of the very finest particles of dust. The larger particles fall very rapidly. In fact, so rapidly as to be buried towns. We may suppose the same is true of the sea salt in the air. The finest particles may be carried to great heights than the larger ones, provided they escape the rain long enough to reach the ground in the first place. The volcanic dust is thrown rapidly to the great height. The dust is thrown into the air at the sea level and reaching a great height is very for-

Therefore, we may suppose that the absence of goiter in mountainous regions is due to the deficiency hypothesis. The absence of iodine from the rain water and soil in mountainous regions would necessitate its absence from the food growing in the region and the animals subsisting entirely upon the plants and animals. Man, however, may receive considerable food from some distance. Food rich in iodine, such as fish, oysters, squid, sea urchin ovaries and sea weed, is consumed to a much greater extent along the sea coast than in inland regions. Sea weed is not a normal article of diet and is only eaten occasionally by the Japanese and certain other people living close to the sea. Sea food, due to its perishable nature, is largely consumed close to the sea. Therefore, even with considerable means of food distribution, the absence of goiter to distance from the sea can still be maintained. Water might hold some relations. Water flows toward the coast and therefore does not bring iodine from inland regions richer in it. Water courses rise either in mountainous regions or in inland lakes and these are goiterous regions. Certain mineral waters may be exceptions but the consumption of such mineral water is rather limited. The principal other factor in the diet is salt. Salt was first obtained by the evaporation of sea water. The process used reduces the amount of iodine, but the extent of reduction depends upon the amount of refining that the salt undergoes. The sea water is evaporated in shallow ponds until the calcium carbonate precipitates. It is then further evaporated in other ponds until the sodium chloride

crystallizes out. The mother liquor from the sodium chloride crystals, known by geologists as the bittern, contains most of the iodine along with magnesium chloride and other salts. This crude sodium chloride, which may have some iodine clinging to it, was formerly consumed in this condition but nowadays is often further purified by washing and recrystallization so that the iodine, which is in very low concentration in the sea water, is reduced to infinitesimal quantities. Salt was not purified to as great an extent in the early days as it is now. When it comes to rock salt Nature has already purified it to some extent. Van't Hoff showed the mechanism of stratification of the rock salt deposits. The sodium chloride layers are already more or less purified. This salt when it is mined in the dry state or when it is obtained from salt springs, which consist of water which has come in contact with these salt deposits, is still further purified for table use. Hayhurst¹⁴ investigated some of the salt works in Ohio where the salt is obtained from deep wells. Bromine and a trace of iodine are separated out of the salt and the bromine sold as a by-product.

I have been unable to obtain any evaporated sea water, that is to say, salts obtained from the sea water without fractional precipitation or purification, from any commercial salt manufacturers on the coast. Through the kindness of Metz & Company, Dr. Sherndahl evaporated 100 gallons of sea water for me to use in experimental feeding. This, together with sea water which I have had opportunity to evaporate, has been dried by baking it in an oven. When the last traces of water are eliminated in this way, hydrochloric acid fumes are also given off. The cause of this, as pointed out by Sorensen, is a reaction between magnesium and the other salts whereby oxides of the alkaline earth metals are formed with the elimination of hydrochloric acid. If the baking is continued long enough no calcium or magnesium chloride remains and therefore the salt remains dry. If the sea water has been evaporated in an iron kettle some iron oxide is added to it, which improves it from a nutritive standpoint. The necessity of baking may be

¹⁴ Hayhurst, E. R., SCIENCE, 1921, liv: 131.

eliminated by adding 6 grams of H_3PO_4 to the liter and this salt may aid in the treatment of rickets. In my animal experiments this evaporated sea water has been used for generations of animals as the salt ration, with gratifying results. It is very low in phosphoric acid unless H_3PO_4 has been added, and if casein is used as the protein there is not sufficient phosphoric acid in the casein for the nutritional requirements. The question as to whether there is sufficient calcium or not for the total calcium ration has not been definitely settled. If wheat flour is used for the carbohydrate portion of the ration there is sufficient additional calcium in the wheat flour to bring the calcium up to the requirements.

The question arises whether it would not be advisable for us to feed our children an impure salt. If iodine is the only mineral constituent that might be deficient it could be easily added to the salt. We have not proved, however, that the other mineral constituents of sea water are not necessary in the diet. Therefore, it would seem much simpler to use evaporated sea water as the salt ration if it could be obtained, and it only remains to create a demand for it. The present process of com-

mercial evaporation of sea water could be simplified if an impure salt was desired. In other words, is to say, only one pond would be needed for the evaporation of the sea water. The water could be evaporated in this pond as practical by the sun. The total content of this pond, including both solids and liquids, could then be removed and evaporated in a large pan and thoroughly mixed, and baked at a high enough temperature to produce a dry salt. In case the crystals of salt were large, owing to the slow evaporation at first, they could be broken up by grinding. In baking, however, there is a tendency for these crystals to break up. The inclusion of a little earth with the salt would not impair its nutritive qualities and the product would be sterilized by the high temperature used in baking. It has been suggested that salt obtained by the usual method from the salt evaporating plants on the French coast is reeking with bacteria. The production of a sterile product might be an advantage. The dietary salt of several adults, children and infants has been limited to the above diet from Metz for many months with gratifying results, in a goiterous region.

CHART FOR THE CONVERSION OF COLORI- METRIC READINGS INTO HYDROGEN ION CONCENTRATION

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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. LIV, No. 3, NOVEMBER, 1922

CHART FOR THE CONVERSION OF COLORIMETRIC READINGS INTO HYDROGEN ION CONCENTRATION.

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Medical School, Minneapolis.)

(Received for publication, May 29, 1922.)

The most popular method of determining hydrogen ion concentration by the use of indicators has been the method of making buffer mixtures of known hydrogen ion concentration and adding indicators to them; at the same time adding the same quantity of indicator to the same volume of the unknown mixture and noting the buffer mixture that matched the unknown in color. This method necessitated knowing two things: one was the hydrogen ion concentrations of the buffer mixtures and the other was the difference in salt error between the buffer mixture and the unknown. In the practical working of the method other difficulties arose: (1) errors due to concentration of the indicator, since the same quantity of indicator had to be added to the buffer mixture and to the unknown, and (2) errors due to impurities in the indicator. These errors were difficult to avoid when the buffer mixture was sealed up and kept for a long time after the indicator had been added. If, however, the indicator is added to the standard at the same time it is added to the unknown, such errors can easily be avoided since the same sample of indicator can be used in adding portions to the unknown and to standard solutions. Perhaps on account of the above difficulties a number of persons have recently been using methods based on the percentage of the indicator that is in the so called dissociated condition.

We do not wish here to go into the theory of the color production, but take the simple hypothesis of Ostwald that the free indicator is undissociated and the salt of the indicator is largely, and under certain conditions, 100 per cent dissociated. Perhaps the simplest application of this method is the use of indicators which are

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colorless in the undissociated condition, such as phenolphthalein, and compare the colors in a Duboscq colorimeter as follows: Equal volumes of unknown solution and of distilled water that has been made alkaline are taken, and to each of these the same quantity of indicator is added. In the alkaline-distilled-water-solution it is presumed that 100 per cent of the indicator is dissociated or in the red condition. This is tested by adding more alkali until the indicator solution does not become any redder. The unknown solution with indicator is placed in the left-hand cup of the colorimeter and this cup set at some known point, such as 10 mm., between the plunger and the bottom of the cup. The alkaline solution of the indicator is added to the right-hand cup and the plunger is raised and lowered until a color match is obtained. The reading on the right millimeter scale, if multiplied by 10, will give the percentage of the indicator that is dissociated in the left-hand cup. It remains to interpret this in terms of hydrogen ion concentration, which is done by the following formula:

$$\alpha = \frac{K}{K + [H^+]}$$

α is the degree of dissociation, K is the dissociation constant, and $[H^+]$ is the concentration of hydrogen ions.

Such calculations, however, although they are done easily by many workers, are only accomplished with difficulty by others, and often the liability for error is proportional to the difficulty. It therefore seems desirable to use the graphic method of working out this formula. Clark, in his book on determination of hydrogen ions, has drawn the graphs for a number of indicators, showing the relation of hydrogen ion concentration to percentage dissociation. These graphs are curves which are not easily drawn by everyone, and, therefore, it seemed desirable to change the graph paper in such a way that the curves would become straight lines. This cannot be done with any of the ordinary graph papers in use, and when it is done the different regions of the graph have different ratios of numerical value to actual dimensions, and some slight error may arise in interpolation between the lines on the graph. Such errors, however, should be small and, therefore, it seemed desirable to publish the accompanying graph with tentative values for a number of indicators. The diagonals

represent the same functions as the curves in the charts in Clark's book and therefore should be easily understood by anyone familiar with hydrogen ion determinations.¹

We add phenolphthalein, for instance, to the unknown solution coming within its range placed in the left-hand cup of the Duboscq colorimeter and to alkaline water in the right-hand cup. If the left-hand cup is set at 10 mm. and the right-hand cup adjusted so as to obtain a color match, the reading on the right-hand scale multiplied by 10 will give the percentage dissociation of the indicator in the unknown solution. This percentage dissociation is then transferred to the chart and its coordinate following to diagonal for phenolphthalein. The point of intersection will give on the abscissa the pH value (or logarithm of the reciprocal of the hydrogen ion concentration). The same principle can be used with thymolphthalein, α -naphtholphthalein, and related indicators. In case of the sulfonic acid compounds of these indicators the Duboscq colorimeter, as ordinarily used, will read with difficulty, owing to the fact (with phenol red, for instance) that the unknown solution in the left-hand cup will show orange, which is a mixture of red and yellow, whereas the right-hand cup will show only different intensities of red as the cup is moved up or down. The eye cannot evaluate the red in the orange without the use of a color screen which would absorb the yellow. The color screen would be placed between the eye and the eyepiece of the colorimeter.

Another method would be to use a color screen absorbing the red or to use monochromatic light in illuminating the apparatus. Suppose we use sodium light. This would pass through the yellow solution and yet it would be absorbed by the red and hence the red alone or the red element in the orange would appear dark and a matching of the two sides would be a matching of the intensity of monochromatic light.

¹ See Clark, W. M., *The determination of hydrogen ions*, Baltimore, 1920. The equation on which the chart is based is No. 7, p. 20:

$$\text{pH} = \log \frac{1}{[\text{H}^+]} = \log \frac{1}{K} + \log \frac{\alpha}{1 - \alpha}.$$

Values of $\log \frac{\alpha}{1 - \alpha}$ are given on p. 306.

A simple method of matching these sulfonic acid dyes, such as phenol red, has been used by Barnett and Barnett² and fully described by them. The principle of this is a rectangular trough of glass, divided diagonally by a vertical sheet of glass into two compartments. The equal volumes, say 100 cc. of unknown and of two distilled water solutions, are carefully measured out and the same quantity of indicator added to each. One of these distilled water solutions is made acid until the indicator is all in the yellow form and the other made alkaline until the indicator is all in the red form and the yellow solution is added to one compartment and the red to the other. If we then look through the trough in the horizontal direction, the color shades from yellow at one end through various shades of orange to red at the other end. In another rectangular trough, made the same thickness as this one, the unknown is placed. The unknown is then moved back and forth above the former and matched with some portion of the long trough. This point of matching is then measured in percentage of the total length, beginning at the yellow end; that is to say, the percentage red is determined in this way. With this value of percentage red (percentage dissociation) we may use Chart 1 as we did with phenolphthalein.

Victor Myers³ has demonstrated a double-wedged colorimeter built on the same principles as this simple trough and the values obtained with it may be converted into pH values by Chart 1.

Another apparatus that has been used is a Duboscq colorimeter with two cups on the right-hand side, one movable and working inside the other. This has been described with detailed instructions as to its use.⁴ One of these instruments was made under the direction of E. P. Lyon and used considerably, the values being interpreted by means of Chart 1 and compared with values obtained by the use of buffer solutions. The method is simply the placing of the unknown in the left-hand cup (colored with phenol red, for instance) and taking the red solution out of Barnett's apparatus and placing it in the inner cup and the yellow solution out of Barnett's apparatus and placing it in the outer cup. The outer

² Barnett, G. D., and Barnett, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1920-21, xviii, 127.

³ Myers, V. C., *J. Biol. Chem.*, 1922, 1, p. xxii.

⁴ Gillespie, L. J., *J. Bact.*, 1921, vi, 399.

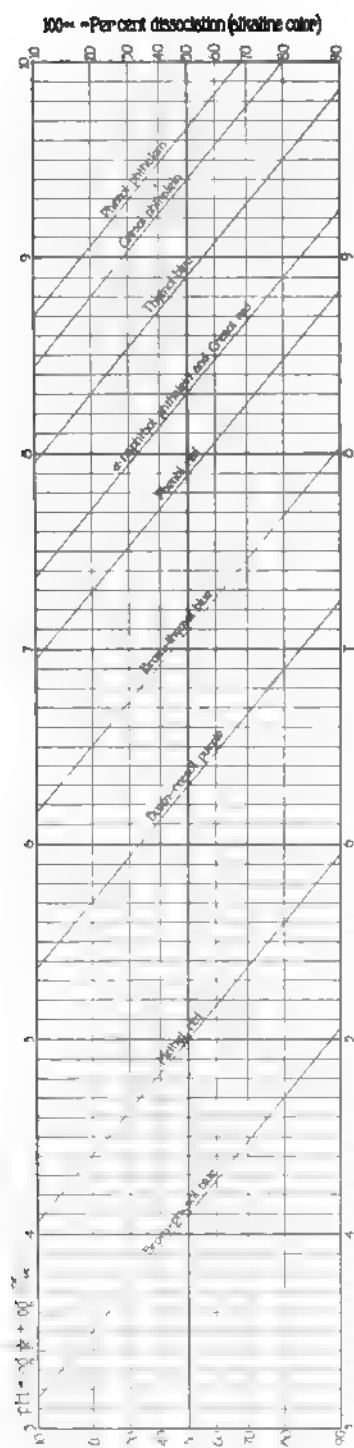


CHART 1.

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cup and the plunger are fixed and only the inner cup is movable. The scale on the right-hand side measures in millimeters, but applies to the movement of the inner cup only. The outer cup is fixed the same distance from the plunger as the left-hand cup, namely 10 mm. in this case. The unknown will show an orange color. By moving the inner cup up and down an exact color match will be obtained. When the cup moves upward the red is decreased and the yellow increased and when it is moved downward the red is increased and the yellow decreased.

The chief value of Chart 1 probably rests in the ease with which new data may be added to it. For instance, let us suppose that we have an indicator that is not on the chart. We first determine its dissociation constant (K). If we take the logarithm of the reciprocal of the dissociation constant and find its numerical value on the pH scale and make a mark where it crosses the 50 per cent coordinate, and draw through this point of intersection a diagonal line parallel to the diagonals on the chart, we will have a "curve" for converting values obtained with this indicator into pH values as has been described. Some samples of indicator may show dissociation constants different from those of other samples and new diagonal lines for these new samples may be made in this same manner. Also the presence of neutral salts may change the dissociation constant of an indicator and in this way a new diagonal for a different salt concentration may be drawn. It is not even necessary to know the dissociation constant. If we can determine the dissociation at any pH value and fix this point on the chart and draw a diagonal through this point parallel to the diagonals, we have the values for the new indicator or for any indicator under new conditions.

The different regions of the chart are not of the same sensitiveness so far as the detecting of the color change is concerned. The eye is subject to Weber's law, that is to say, the eye detects a percentage change in a color, and the absolute change it can detect is less the greater the intensity of the color. For this reason the portion of the chart showing less than 50 per cent dissociation is more sensitive than that showing more than 50 per cent. In case of methyl red, however, the reciprocal of the dissociation was used in drawing its curve in order to obtain the same slope of the curve as in the case of acid dyes. This will lead to no error

in practical application since the same conventions in the acid and alkaline solutions of the dye will be used, but the portion of the chart that is marked for more than 50 per cent dissociation would be the most sensitive region for methyl red.

Notes Added to Proof.—According to Michaelis and Gyemant,^{*} the curve for phenolphthalein is not quite a straight line as in the chart. Their figures for 100 α and pH are as follows: 10, 8.95; 20, 9.20; 30, 9.35; 40, 9.50; 50, 9.70; 60, 9.90; 70, 10.10; 80, 10.30. They also add the following useful indicators with the pH at $\alpha = 50$ per cent; 2,6-dinitrophenol, 3.69; 2,4-dinitrophenol, 4.06; *p*-nitrophenol, 7.18; *m*-nitrophenol, 8.35. They made these determinations at 18° and they found that rise in temperature moves the figures to the acid side; that is to say, the values for pH decrease slightly.

^{*} Michaelis, L., and Gyemant, A., *Biochem. Z.*, 1920, cix, 165.



THE WAVERLY
BALTIMORE, U.

SIMPLE GOITER AS A RESULT OF IODIN DEFICIENCY

PRELIMINARY PAPER *

J. F. McCLENDON, PH.D.

WITH THE TECHNICAL ASSISTANCE OF

AGNES WILLIAMS, B.A.

MINNEAPOLIS

Chatin¹ advanced the hypothesis that simple goiter is correlated with a low iodine content of drinking water, but his opinion was not accepted. Heretofore the methods of iodine analysis have not been very well developed so as to determine small quantities in water. According to the director of the Geological Survey, iodine has been detected in waters containing 1,000 parts per million of chlorine and quantitatively in mineral waters containing 5,000 parts of chlorine per million. In Europe, certain determinations of the iodine content have been made; for instance, Macadam determined the iodine in 100 gallons of the Edinburgh water supply.

We have been using the method that I described before the Society of Biological Chemists in Toronto, Dec. 28, 1922, and our results so far have all fallen into line with the idea that there is an inverse ratio between the amount of iodine in surface waters or those of shallow wells or springs, and the distribution of goiter. The accompanying map shows the general findings. The goiter data were taken from photostat copies of the manuscript of "Defects Found in Drafted Men," War Department, 1920, and also from Table xxxiii of that volume. This represents the examination of about two and one-half million drafted men, and yet this number was too small for accurate statistical work in relation to simple goiter. The probable error was very high, and in interpreting these data a certain amount of smoothing of the curves was done in order

* From the Laboratory of Physiological Chemistry, University of Minnesota.

1. Chatin Compt. rend. Acad. de sc., 1850

to make the zones continuous. The probable error is high, owing not only to the small number of cases of goiter reported, but also to the fact that different examiners may have different standards. If we compare the data by the draft board with that by Kerr² at Camp Lewis, we notice perhaps the greatest possible difference. Kerr finds 390 enlarged thyroids to each thousand recruits from the state of Washington, and the draft board finds only forty cases of simple goiter³ to each thousand men in the county in which the highest incidence of goiter was noted in Washington. As a matter of fact, the zones would probably not be continuous if the people remained in small localities and ate the food and drank the water from those localities.

For instance, in olden times in Europe, particularly in the Alps, according to many observers, and in the Himalaya Mountains, according to McCarrison,⁴ it was often possible to find two villages very close to each other in which the incidence of goiter is very different. In the United States, however, owing to migration of the population and transportation of food and drink there has been a smoothing out of the areas and obliteration of local differences. In fact, it is doubtful whether we are warranted in making four zones; perhaps three or even two are about all that are warranted. Two contiguous zones would necessarily shade into each other by migration. The goiter incidence in the different zones is quite distinct. In the first zone we find from 15 to 30 to each thousand men; in the second zone, from 5 to 15; third zone, from 1 to 5, and fourth zone, from 0 to 1.

The amount of iodine in drinking water from the few analyses so far completed shows in parts per billion in the first zone, from 0.01 to 0.1; second zone, from 0.015 to 1.2; third zone, from 0.06 to 9, and fourth zone, from 1.4 to 10. Of course, certain mineral waters are excluded from these analyses.

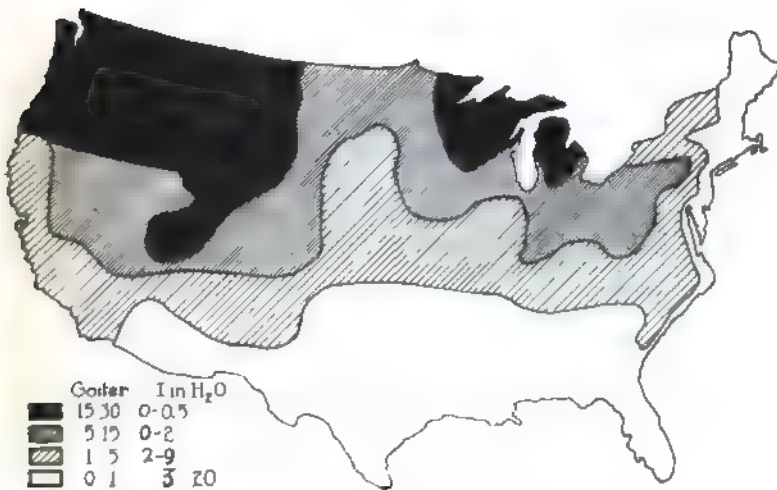
It is not intended to intimate that there is enough iodine in drinking water to prevent goiter. Even in

2. Kerr, W. J.: Preliminary Survey of Thyroid Gland Among 2,182 Recruits at Camp Lewis, Washington, *Arch. Int. Med.* **24**: 347 (Sept.) 1919.

3. Too long to wear a military collar.

4. McCarrison, Robert: *The Thyroid Gland in Health and Disease*, New York, William Wood & Co., 1917.

case of the water of the lower Mississippi and certain well waters that contain around 10 parts per billion of iodine, 10 liters (quarts) of water would have to be drunk before 0.1 mg. of iodine was ingested. According to modern Swiss practice, 0.1 mg. of iodine a day is about the dose recommended for schoolchildren for the prevention of goiter, and it is certainly impossible for the schoolchild to drink 10 liters of water a day. Furthermore, in the second zone the incidence of goiter is reduced very much from the first zone, and it is enormously less than in the very goitrous regions of Switzerland; and yet in some regions the iodine in the water is less than 0.1 mg. in 100 liters.



Comparison of iodine in water supplies and distribution of goiter: iodine in parts per billion of representative rivers, goiter rate per thousand from Table xxxii, Defects Found in Drafted Men, War Department, 1920, and manuscript; curves smoothed.

We would then have to suppose that the amount of iodine in the daily consumption of a few liters, say 0.003 mg. or less, showed some protective action. It seems more probable that the iodine in water is merely an indication of the iodine in soils which come in contact with this water, and that the iodine in soils is concentrated by the plants growing in the soils, and in that way the population over a given area receives iodine through the food. Therefore, water which is taken

from the very shallow wells or from the run-off of unpopulated areas would probably be the most typical. The amount of iodine in rivers is significant only in case the volume of the water is very large in proportion to the population along its banks, since it is very probable that all of the iodine given as drugs, besides all that taken in as food, finally finds its way into these rivers through sewage. The amount of iodine exported from Chile in 1904 was 500 metric tons. It is probable that a fair portion of this reached the United States. The amount of sewage contamination, however, could probably be measured by its chlorine content, and therefore any greatly contaminated waters could be excluded from the tables. This is evidenced by the fact that about 7,000,000 tons of salt are used in the United States each year.

As to the cause of this unequal distribution of iodine in natural waters and therefore possibly in the soil, there are at least three factors at work. In the first place, iodine is found in igneous rocks, and in the weathering of these rocks some iodine is liberated. Since the weathering of igneous rocks is very slow, the iodine content of water in contact with them would be very small. This is probably the case in western Oregon, which is largely covered by basalt in such a manner that the surface water or the roots of plants have no access to any iodine except that derived from the igneous rocks. Most of the iodine in the world is in the sea, which contains about 60 billion metric tons of it. Its concentration in the sea has probably been determined with some accuracy, since recent investigators agree pretty well on this point. Winkler⁵ found 0.038 mg. of iodine in a liter of sea water. Cameron⁶ found about the same amount. I⁷ was able to confirm Winkler's work in a rough manner, finding about 0.04 or 0.05 mg. of iodine in a liter of sea water. This is about 100 times as concentrated as in water from Zone 1.

Much of the United States at intervals from the Cambrian period to the Pliocene period has been submerged under the sea, and in that way has been soaked in sea water for ages, thus receiving a store of iodine;

5. Winkler: *Ztschr. f. angewandte Chem.* 20: 205, 1916.

6. Cameron. *Contribution to Canadian Biology*, University of Toronto Press, 1922, p. 75.

7. McClelland, J. F.: *Science* 56: 269, 1922.

but, as the land emerged from the sea, this iodine was gradually leached out by the rains, except when buried 100 or more feet deep, as in salt deposits in Michigan, New York and Ohio. The region low in goiter, in the Southern states, was the only large region that was submerged beneath the sea in a late geological period (Pliocene), and the amount of chlorine in many of the waters, in the waters of Texas, for instance, indicates that the sea salts have not entirely leached out of the surface in this region. A salt lake covered Kansas and the adjacent territory during the Permian period.

The third factor in the iodine distribution is atmospheric iodine from sea spray and the burning of coal. The United States Geological Survey has carefully mapped the amount of sea water contaminating rain water, and finds that all of the chlorine in the rain water of New York and New England is due to sea spray. The quantity decreases rapidly as we go from the coast toward the Great Lakes, and it may be noticed from the accompanying map that the amount of goiter increases from the coast toward the Great Lakes. The iodine thus brought in amounts to 0.012 part per billion of rain water three miles from the coast, and decreases to about 0.0004 part per billion in the region of Lake Erie and Lake Ontario. This is added to the iodine derived from the soil, and may be significant. The effect of sea spray on the western coast is not shown by the draft board examinations in the prevalence of goiter. This is probably due to the obliteration of this beneficial effect by migration. The younger the person, the less time he has had to migrate; therefore we should expect more localized conditions to be reflected in infants and children. In fact, a map made by Dr. D. C. Hall, professor of hygiene, University of Washington, shows that the Puget Sound region is a region low in goiter among schoolchildren. As we recede from the sound or go down to the narrow portion, goiter increases. Narrow bodies of water have not much effect in throwing sea spray into the air, as is shown by the fact that whereas the widest portion of Long Island Sound throws considerable sea spray into the air, the narrow portion of it does not. It is very probable that the schoolchildren all along the western coast are

comparatively free from goiter. Besides the sea spray, the spray of Great Salt Lake contains considerable iodine, as I was able to find 0.07 mg. of iodine per liter in it. This is evidenced on the accompanying map by an adjacent region of low goiter incidence, as contrasting with the high goiter incidence in eastern and southern Utah.

The only way to make certain of this relation between goiter and iodine is to make a large number of analyses of water from different parts of the country. Owing to the large size of the sample required, it is impossible for one investigator to procure them by his own efforts. It is therefore hoped that the interested parties will send in samples, in return for which analyses will be sent them.

The method for collecting the sample is as follows :

One-half teaspoonful of soda is placed in a tin dish pan. Several gallons of water are added, and are evaporated over a brisk fire. Measured quantities of water are added until the required amount for the zone has been added. The entire amount should be evaporated to less than a quart, and filtered into an evaporating dish and evaporated to dryness. The residue in the evaporating dish (but not that in the dishpan) is scraped out and sent to me. The smallest size sample required for Zone 1 is 25 gallons; Zone 2, 15 gallons; Zone 3, 10 gallons; Zone 4, 5 gallons. Larger samples give more reliable results.

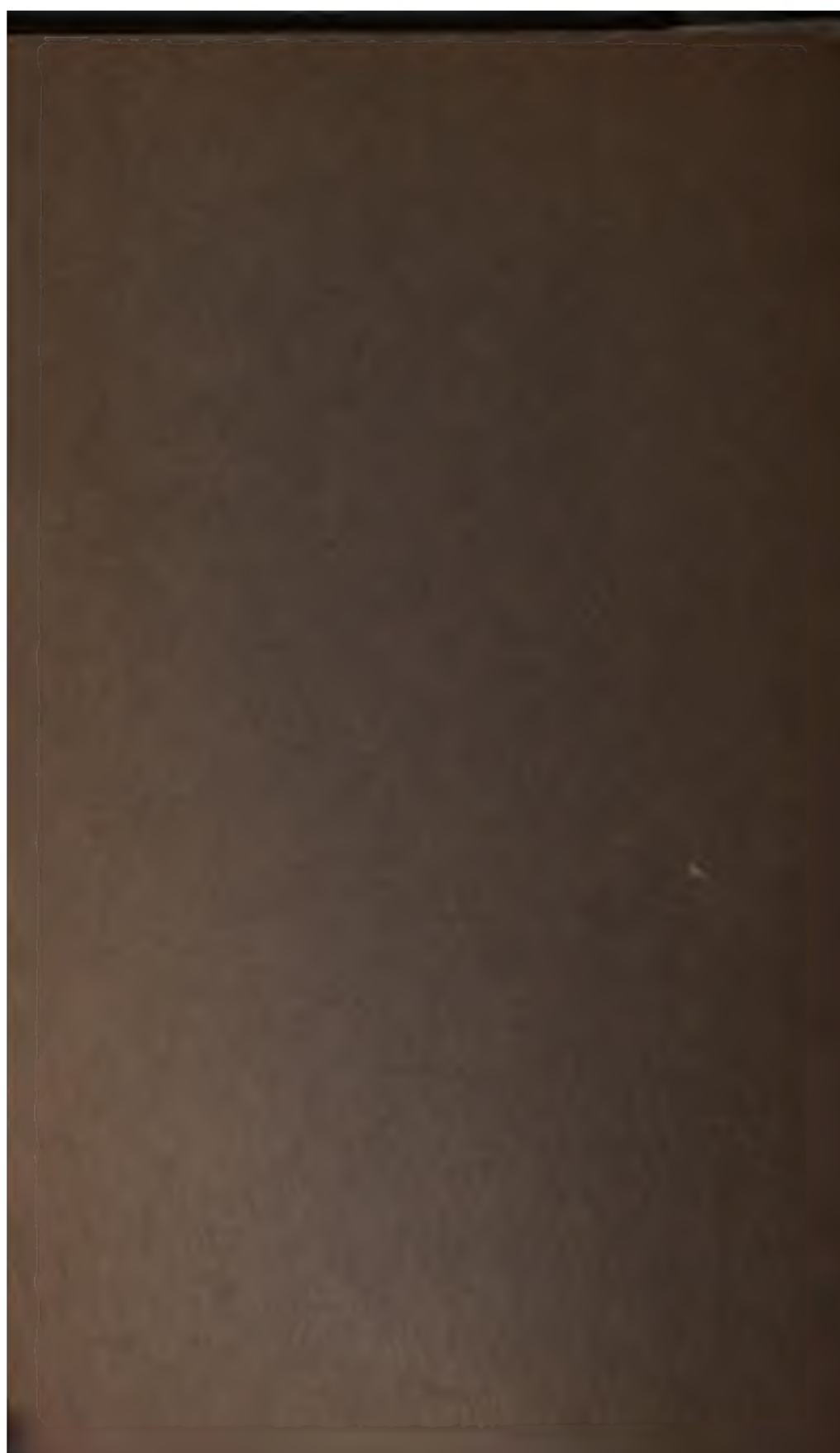
*Reprinted from The Journal of the American Medical Association
March 3, 1923, Vol. 80, pp. 600 and 601*

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